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by Improved Water Soluble Taxanes

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<b>13. Abstract (Maximum 200 Words)</b> The central objective of this investigation was to synthesize and test a novel class of water soluble taxol analogs for potential application in breast cancer chemotherapy, with particular emphasis on overcoming the problem of taxol-resistance that frequently encountered in clinical therapy. We hypothesized that the proposed novel taxol analogs containing both effective functional groups at side chain and strong water soluble moieties at C7 position should be able to circumvent P-glycoprotein mediated multidrug resistance and to enhance the effectiveness in killing taxol-resistant breast cancer cells. We have made significant progress in this research project. Three key intermediate compounds have been successfully synthesized and identified, whereas the synthesis of the proposed taxol analogs is nearing completion. The final intermediate compound has also been made and detected. The feasible and practicable synthetic routes have been established in this project for preparation of these extremely important intermediate compounds that will essentially be used for the successful synthesis of the proposed water soluble taxanes. All these encouraging results will greatly contribute to the development of water soluble taxol analogs for overcoming taxol-resistance in human breast cancers. If this project is successful, the clinical benefits to breast cancer patients could be enormous.				
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## I. INTRODUCTION

Paclitaxel (taxol) and docetaxel (taxotere) are new antitumor agents developed during the past decade (1-2). Both taxol and taxotere are believed to act as antimicrotubule agents that disrupt the function of microtubules by shifting the equilibrium between soluble tubulin dimers and polymerized microtubules, thereby stabilizing microtubules (1-3). Clinical trials have shown impressive activity of taxol and taxotere in human breast cancers (4). However, toxic side effects of taxanes on normal tissues, acquired multidrug resistance following taxane drug treatment, and their poor water solubility limit their clinical applications in breast cancer therapy (5-8).

The central objective of this investigation was to synthesize and test a novel class of water soluble taxol analogs for potential application in breast cancer chemotherapy, with particular emphasis on overcoming the problem of taxol resistance that is frequently encountered in clinical therapy. We proposed to perform: (a) synthesis of three novel taxol analogs containing nitro and O-tert-butyl groups at side chain and also a polyamine (spermine or spermidine or putrescine) at C7 position and their salts; (b) evaluation of chemotherapeutic effects of novel taxol analogs on two pairs of sensitive or resistant human breast cancer cell lines (MCF-7: estrogen receptor positive [ER+], taxol-sensitive; MCF-7/ADR [NCI/ADR-RES]: ER-, taxol-resistant, P-glycoprotein positive [Pgp+]; MDA-MB-231: ER-, taxol-sensitive; MDA-MB-453: ER-, taxol-resistant, HER-2/neu overexpression, Pgp-) *in vitro* tissue culture; (c) Investigation of the *in vivo* efficacy of the most effective analog in nude mice bearing two pairs of sensitive or resistant breast cancer cell xenografts; (d) toxicity of the most promising analog in mice; (e) molecular mechanism studies on MDR gene expression and function. As shown below, we have made significant progress in our research project during the period of September 1, 2000 - September 1, 2001.

## II. SYNTHESIS OF TAXOL ANALOGS

The study of synthesis of three proposed taxol analogs has been performed. The synthetic procedures for preparation of these new and complex compounds involved multiple steps and production of several key reaction intermediates. The first proposed synthetic route we designed and tested included the synthesis of 2'-triethylsilyloxy-taxol, 2'-triethylsilyloxy-7-chlorated acetyl-taxol, and 2'-triethylsilyloxy-7-polyamine-acetyl-taxol, and hydrolysis of 2'-triethylsilyloxy-7-polyamine-acetyl-taxol. Reaction of triethylsilicic chloride and taxol in pyridine produced 2'-triethylsilyloxy-taxol in resulting mixture. Purification of the crude mixture by flash chromatography gave solid pure 2'-triethylsilyloxy-taxol in a yield of 65%. The second intermediate compound, 2'-triethylsilyloxy-7-chlorated acetyl-taxol, was also synthesized using 2'-triethylsilyloxy-taxol as a reaction intermediate. The removal of the organic solvent gave in a yield of 76%. The coupling reactions of 2'-triethylsilyloxy-7-chlorated acetyl-taxol and a polyamine (spermine or spermidine or putrescine) have failed many times to give target compounds. The reasons for the failure of the coupling reactions could be due to limited reaction activity of this coupling intermediate compound, 2'-triethylsilyloxy-7-chlorated acetyl-taxol. Therefore, there was a great need for the synthesis of the optimal coupling

intermediates with even higher reaction activity at C7 position of taxane core structure to which a polyamine could efficiently be attached.

The modified synthetic routes for making water soluble taxane analogs included the synthesis of 2'-triethylsilyloxy-taxol, 2'-triethylsilyloxy-7-imidazolidetaxol, and 2'-triethylsilyloxy-7-polyamine-amido-carbamate-taxol, and hydrolysis of 2'-triethylsilyloxy-7-polyamine-amido-carbamate-taxol. The intermediate compound obtained from previous synthetic reaction, 2'-triethylsilyloxy-taxol, reacted with carbonyldiimidazole in  $\text{CH}_2\text{Cl}_2$  solvent, to yield 2'-triethylsilyloxy-7-imidazolidetaxol (72%). To a solution of 2'-triethylsilyloxy-7-imidazolidetaxol in  $\text{CH}_2\text{Cl}_2$ , excess amount of spermine was added under nitrogen. The mixture was stirred at room temperature for 16 hrs. The reaction mixture was diluted and extracted by ethyl acetate solvent and separated by column chromatography. A very small amount of a new compound produced in this coupling reaction was detected by thin layer chromatography. This new compound could be a putative target molecule. Next steps will be to optimize the reaction conditions (reaction time, solvent, and temperature), to increase the yield of this new compound presumably due to the inappropriate conditions leading to the low yield, to identify the final intermediate compound, 2'-triethylsilyloxy-7-polyamine-amido-carbamate-taxol, and to then complete the hydrolysis of 2'-triethylsilyloxy-7-polyamine-amido-carbamate-taxol. It is encouraging that the key intermediate compounds have been synthesized and we are very close to accomplish the synthesis of our proposed objective taxol analogs. In short, we have made substantial progress in this study. All the results will essentially be used for the successful synthesis of the target taxol analogs and will directly contribute to the development of this novel class of water soluble taxol analogs for improved breast cancer therapy.

### III. KEY RESEARCH ACCOMPLISHMENTS

1. We have successfully synthesized, purified, and analyzed three key intermediate compounds.
2. We have also synthesized the final intermediate compound which could be converted into our proposed taxol analog molecule. The results demonstrated that the synthetic methods for making proposed water soluble taxane compounds could be feasible.
3. We have established the modified synthetic routes for preparation of these extremely valuable compounds with a high yield.

### IV. REPORTABLE OUTCOMES

1. One manuscript has been submitted to the **Journal of Cancer Immunology and Immunotherapy** (see the attached manuscript). One manuscript is in preparation for submission for publication.

2. During this one-year grant period, we have also made two presentations of this research project in a seminar format to St. Mary's Medical Center Residency program and California Pacific Medical Center Research Institute.

## V. CONCLUSIONS

We have accomplished the synthesis, identification, and analysis of the most important intermediate compounds. The synthetic routes for preparation of them have been well established in our laboratory. The final intermediate compound has been synthesized and detected. The studies demonstrated that the approaches and procedures for the synthesis of the proposed water soluble taxanes could be feasible and practicable. This one-year grant has generated large amounts of remarkable results that will be employed for the completion of the synthesis of our proposed target compounds. All the studies will significantly contribute to the development of novel water soluble taxane analogs for greatly improved therapy of breast cancer, with particular emphasis on overcoming the problem of taxol-resistance. It is anticipated that our long-term goal of this proposal can be achieved. If this investigation is successful, the benefit to breast cancer patients could be substantial.

## VI. REFERENCES

1. Rogers BB. Taxol: A promising new drug of the '90's. **Oncology Nursing Forum**, 20: 1483-1489, 1993.
2. Rowinsky EK, Donehower RC. Paclitaxel (Taxol), **New England J. Med.** 332:1004-1008, 1995.
3. Horwitz SB. Mechanism of action of taxol. **TIPS**. 13:134-136, 1992.
4. Holmes FA, Kudelka AP, Kavanagh JJ, Huber, MH, Bjani JA, and Valer V. Clinical trials with paclitaxel and docetaxel. In Georg GI, Chen TT, Ojima I and Vyas DM (eds): **"Taxane Anticancer Agents. Basic Science and Current Status"**. ACS Symposium Series 583, American Chemical Society, Workshop on Taxol and Taxus, Washington, DC, pp. 31-57, 1995.
5. Pratt WB, Ruddon RW, Ensminger WD, Maybaum J. Resistance to anticancer drugs. In: **The anticancer drugs 2<sup>nd</sup> ed.**. Oxford University Press, Inc.: New York, NY, Chapter 4, pp. 50-66, 1994.
6. Cabral RF, Brady RC, Shibler MJ. A mechanism of cellular resistance to drugs that interfere with microtubule assembly. **Ann. N. Y. Acad. Sci.** 466:745-752, 1986.
7. Straubinger RM, Sharma A, Sharma US, and Balasubramanian SV. Pharmacology and antitumor effect of novel paclitaxel formulations. In Georg GI, Chen TT, Ojima I and Vyas DM (eds): **"Taxane Anticancer Agents. Basic Science and Current Status"**. ACS Symposium Series 583, American Chemical Society, Workshop on Taxol and Taxus, Washington, DC, pp. 111-123, 1995.
8. Vyas DM, Ueda Y, Wong H, Matiskella JD, Hauck, S, Mikkilineni AB, Farina Vittorio, Rose WC, and Casazza AM. Phosphatase-activated prodrugs of paclitaxel. In Georg GI, Chen TT, Ojima I and Vyas DM (eds): **"Taxane Anticancer Agents. Basic Science and Current Status"**. ACS Symposium Series 583, American



Chemical Society, Workshop on Taxol and Taxus, Washington, DC, pp. 124-137, 1995.

## **Anticancer Effects and Mechanisms of Polysaccharide-K (PSK): Implications of Cancer Immunotherapy**

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## **Abstract**

Polysaccharide-K (polysaccharide-Kureha; PSK), also known as krestin, is a unique protein-bound polysaccharide, which has been used as a chemoimmunotherapy agent in the treatment of cancer in Asia for over 30 years. PSK and Polysaccharopeptide (PSP) are both protein-bound polysaccharides which are derived from the CM-101 and COV-1 strains of the fungus *Coriolus versicolor* by Japanese and Chinese researchers, respectively. Both polysaccharide preparations have documented anticancer activity in vitro, in vivo, and in human clinical trials, though PSK has been researched longer and has therefore undergone more thorough laboratory, animal and clinical testing. Several randomized clinical trials have demonstrated that PSK has great potential as an adjuvant cancer therapy agent, with positive results seen in the adjuvant treatment of gastric, esophageal, colorectal, breast, and lung cancers. These studies have suggested the efficacy of PSK as an immunotherapy or biological response modifier (BRM). BRMs potentially have the ability to improve the "host versus tumor response," thereby increasing the ability of the host to defend itself from tumor progression. The mechanisms of biological response modification by PSK have yet to be clearly and completely elucidated. Some studies suggest that PSK may act to increase leukocyte activation and response through upregulation of key cytokines. Indeed, natural killer (NK) and lymphocyte activated killer (LAK) cell activation has been demonstrated in vivo and in vitro, and recent genetic studies reveal increased expression of key immune cytokines in response to treatment with PSK. An antimetastatic action of PSK has also been demonstrated and is perhaps attributed to its potential to inhibit metalloproteinases and other enzymes involved in metastatic activity. PSK has also been shown to cause differentiation of leukemic cells in vitro, and this effect has been attributed to induction of differentiation cytokines. PSK has also been shown to have antioxidant capacity which may allow it to play a role as a normal tissue chemo- and radio- protector when used in combination with adjuvant or definitive chemotherapy and/or radiotherapy in the treatment of cancer, and may also enable it to defend the host from oxidative stress. Interestingly, studies have also shown that PSK may actually inhibit carcinogenesis by inhibiting the action of various carcinogens on vulnerable cell lines. This action of PSK may play a role in preventing second primary tumors when an inducing agent, such as tobacco or asbestos is suspected, and may also prevent second malignancies due to the carcinogenic effects of radiotherapy and cytotoxic chemotherapy. Another very important aspect of chemoimmunotherapy, in general, is that it may be used on debilitated patients such as those with AIDS and the elderly who might otherwise be denied potentially helpful adjuvant cytotoxic chemotherapy. Further determination of the mechanisms of these anti-cancer, immunostimulating, and biological response modifying effects of PSK as well as other protein-bound polysaccharides is certainly warranted. Indeed, with modern cellular and molecular biology techniques, a better understanding of specific molecular effects of PSK on tumor cells as well as leukocytes may be determined. Much of the research that has been done on PSK is outlined in this paper and may serve as a foundation toward determining the mechanisms of action of this and other protein-bound polysaccharides in the treatment of cancer. This information may open new doors in the development of novel strategies for the treatment of malignancies using

adjuvant immunotherapy in combination with surgery, chemotherapy and/or radiotherapy.

**Key words:** polysaccharide, PSK (krestin), immunotherapy, anticancer, biological response modifier (BRM)

## **Introduction**

Immunotherapy has gained popularity as an adjuvant therapy for cancer during the last 2-3 decades. Numerous approaches utilizing various forms of immunotherapy have been developed, including active nonspecific immunotherapy, adoptive immunotherapy, monoclonal antibody therapy, and active specific immunotherapy [92]. Active nonspecific immunotherapy aims to augment the body's natural immune response without directing it against any specific tumor antigen. Some of the many non-specific immunostimulants developed so far include OK-432, BCG, levamisole, diphtheria toxoid, polysaccharides, and endogenous cytokines such as interleukins, interferons, and colony stimulating factors [1].

The earliest report of a polysaccharide having antitumor activity was in 1943, when "Shear's polysaccharide" was isolated from the bacterium *Serratia marcescens*. Other polysaccharides derived from various bacteria have also exhibited antitumor effects, but most of these bacterial polysaccharides are endotoxic lipopolysaccharides and have undesirable toxic effects [123]. The mushroom *Coriolus versicolor* has been used in traditional Chinese medicine for centuries, and was recorded in the *Compendium of Materia Medica* by Li Shi Zhen during the Ming Dynasty in China as being beneficial to health. Various substances have been isolated from this mushroom. Among them, PSK and PSP have been most widely used and researched [109]. The history of polysaccharides isolated from fungi, as nonspecific immunostimulants, dates back to the 1960s when Japanese scientists began screening polyporaceae and other species for *in vivo* antitumor activity. Numerous extracts from the class Basidiomycetes were found to have antitumor activity in animal models, including one extract derived from *Lentinus edodes* which completely inhibited the growth of sarcoma 180 cells implanted subcutaneously in mice [7]. This polysaccharide called lentinan showed marked antitumor activity, but had only limited oral bioavailability [61]. The search for a similar compound with oral bioavailability and equal or greater antitumor efficacy led researchers at Kureha Chemical Industries to screen over 200 species for antitumor activity [83]. *Coriolus versicolor* was considered to be the most suitable strain due to very high *in vivo* antitumor activity, minimal toxicity, and stability during serial cultivation. In 1971, an active principle was isolated from extracts of cultured hyphae of strain CM-101 through ammonium sulfate precipitation and eventually came to be called polysaccharide Kureha, polysaccharide K (PSK), or krestin [99]. This article aims to summarize remote and recent research findings on PSK, since basic science and clinical research on PSK is the most thorough of any research on any of the plant or mushroom derived protein-bound polysaccharides.

## **Structure and Pharmacological Activity**

PSK is a mixture of compounds, and possesses an average molecular weight of 100 kiloDaltons (kDa). The amino acid composition of the peptide portion constitutes has been roughly determined, as has the polysaccharide portion. The peptide portion constitutes approximately 18-38%, and is relatively rich in the acidic amino acids, aspartate and glutamate, as well as the neutral amino acids, leucine and valine. The structure of the main glycoside portion is  $\beta$ -glucan including  $\alpha$ -1,4 and  $\beta$ -1,3 glucosidic linkages with branched chains including 1 $\rightarrow$ 3, 1 $\rightarrow$ 4, and 1 $\rightarrow$ 6 bonds, with branches at 3- and 6- positions in a proportion of one per several 1 $\rightarrow$ 4 linkages. The primary monomer of the polysaccharide portion is glucose with decreasing percentages of mannose, xylose, galactose and fucose [153]. Also, fractionation of PSK by successive filtration has revealed at least 4 subfractions with molecular weights ranging from <50 kDa to >200 kDa, with the highest molecular weight fraction having the greatest biological activity [133].

Studies of  $^{14}\text{C}$ - and  $^{35}\text{S}$ -labelled PSK have shown that it is partially decomposed in the digestive tract, and small molecular weight digestion products are observed in the serum within two hours of oral ingestion. However, by 4 hours large molecular weight substances consistent with intact PSK are observed in serum. It has therefore been inferred that PSK is absorbed in its large molecular form by pinocytosis, and can exist in this stable state in the blood [153]. PSK is then distributed in bone marrow, salivary gland, brain, liver, spleen, pancreas, and tumor. After 24 hours, 70% of PSK is excreted by expiratory air, and 15-20% is excreted in urine after 72 hours. A small amount is transferred to lymph and bile [45]. Studies using immunohistochemical staining with antibodies to PSK have shown that intratumoral injection of PSK results in phagocytosis of PSK by histiocytes causing them to become antigen-presenting cells [149]. Also, approximately 11.5% of orally administered PSK accumulates in leukocytes of the liver and spleen as demonstrated by anti-PSK antibody staining [171].

With regards to interaction with other drugs, numerous studies have shown that PSK does not effect the pharmacology of other drugs because it does not seem to effect hepatic drug-metabolizing enzymes involved in the chemical processing of most chemotherapy agents [15, 16, 157].

Numerous protein-bound polysaccharides including PSK have been shown to have diverse immunomodulating and anticancer activities [61]. Among the many proteoglycans isolated and tested, those derived from the macrofungi class Basidiomycetes and, to a lesser extent, those from Ascomycetes, seem to have the greatest therapeutic potential due to their minimal toxicity, and potent immunomodulatory and anticancer effects. More specifically, members of the polyporaceae species have traditionally been regarded to have the greatest beneficial effect on the health of cancer patients [150]. The enormous potential variability of polysaccharide structure may allow for tremendous flexibility in the precise regulatory activity necessary for second

messaging in higher organisms (figure 1). Through the study of multiple polysaccharides, several generalities regarding structure have been hypothesized. Most of the antitumor polysaccharides have a basic  $\beta$ -glucan structure with  $\beta$ -1,3-linkages in the main chain and  $\beta$ -1,6-branch points, with relatively short branches. Glucans with high molecular weight and water solubility seem to have the greatest antitumor activity, with some exceptions. Also, a triple-helical structure has been identified as being important for antitumor activity. Finally, chemical modification of various polysaccharides has demonstrated that various biological activities may be enhanced or attenuated, and might be an effective approach to improving the biological activity of these diverse macromolecules [123].

### **Early Clinical Trials**

Early clinical studies were undertaken in Japan, showing significant benefits from the addition of PSK immunotherapy to standard surgery, chemotherapy and/or radiation therapy. Early clinical testing revealed positive results in cervical [31, 139] esophageal [46, 112-113, 120, 137], gastric [17, 29, 40-41, 49-50, 82, 95, 104-107, 111, 144], colorectal [96, 131, 143, 150, 168], bladder [102, 152], nasopharyngeal [10, 20], and lung [44, 114, 117] carcinomas, as well as medulloblastomas [138], astrocytomas [67, 129-130, 164], oligodendrogliomas [66], and leukemias [122]. The early literature also contains numerous case reports suggesting the efficacy of PSK in various other malignancies including sarcomas [34], hepatocellular carcinomas [25, 60, 76, 91, 98, 141, 166], cholangiocarcinoma [77], pancreatic carcinoma [136], renal cell [24, 78], and urethral carcinomas [32], and breast [5] and ovarian [62-63] carcinomas, amongst others, including some reports of partial and complete responses in cases of advanced and even metastatic disease.

Meanwhile, animal studies confirmed the suspected anticancer mechanism of immunostimulation, revealing antibiotic properties of PSK in vivo [93, 160] and in vitro [110]. In addition to ongoing clinical trials in the 1980's, basic science research on the mechanisms of action of PSK continued and further elucidated some of the biochemical actions responsible for its anticancer and immunopotentiating properties. One animal study conducted in the early 1980's confirmed the benefit of administration of PSK and other immunomodulators in combination with radiotherapy, but revealed the significance of appropriate timing of administration of immunotherapy with regard to radiotherapy treatment, suggesting a greater radioprotective benefit when immunomodulators were administered after irradiation [97, 128]. Another study suggested that maximal benefits could be derived from administration of immunopotentiators after administration of cytotoxic chemotherapy [140]. Meanwhile, small clinical trials continued to reveal the utility of PSK as an adjuvant treatment of multiple malignancies when combined with radiation and chemotherapy.

### **Immunomodulatory Effects**

### Early Observations

Early immunological studies suggested that one of the mechanisms of immune enhancement by PSK might be through elevation of serum complement levels in addition to activation of the complement system [57]. Another study suggested activation of the thymus by PSK [156]. Other evidence also suggested enhancement of interferon production by PSK as one of its mechanisms of immunopotential [68]. Subsequent studies revealed that PSK was able to potentiate antiviral activity even in immunodepressed tumor-bearing mice [145], perhaps through lymphocyte activation, but that it did not appear to mediate this effect through enhancement of tumor necrosis factor (TNF) activity [26-27], though this observation has since been revised.

Another immune phenomenon noted in these early studies was that depression of macrophage chemotaxis induced by tumor burden could be alleviated by treatment with PSK [2], and this alleviation of immunodepression was found to be due to restoration of T-cell lymphocyte mediated delayed type hypersensitivity (DTH) [154, 158]. Another early study found that PSK augmented the generation of cytotoxic lymphocytes and the induction of resistance against re-challenge to injected tumor cells in mice. Intraperitoneal injection of PSK—at the site of tumor implantation—was more effective in inducing cytotoxic lymphocytes than oral administration, suggesting that direct contact with PSK caused cellular differentiation of local lymphocytes. Also, PSK was found to increase the threshold number of tumor cells which could be eliminated by immune hosts suggesting importance with regard to elimination of metastasis or local implantation of a limited number of tumor cells [155]. Inhibition of leukocyte migration has also been found to be one of the immunomodulating effects of PSK, perhaps increasing the number of available leukocytes to fight tumor by inhibiting migration away from the tumor site. Also, killer T-cell activity was augmented in tumor-bearing mice by intraperitoneal or oral administration of PSK, and there was a correlation between PSK-associated antitumor effect and killer T-cell activity [161].

PSK has also been shown to influence prostaglandin metabolism, inhibiting the production of thromboxane A<sub>2</sub> (TXA<sub>2</sub>), and stimulating the production of prostacyclin (PGI<sub>2</sub>) [142]. PSK was also found to compete with immunosuppressive substances isolated from the serum of tumor-bearing mice [87-89, 153]. Changes in quantities suppressive factors were also considered to be one of the immunological effects of PSK in gastric cancer patients [115]. One study found that mice exposed to PSK had an increase in a particular serum protein called LC2 which was identified as a serum beta-1-globulin having iron-binding capacity and was considered to be similar to mouse transferrin. This protein was found to restore spleen cell response in tumor-bearing mice, to promote proteose-peptone metabolism in peritoneal macrophages, and to have weak but definite activity against sarcoma-180 cells in vivo [75]. PSK was found to augment the generation of cytotoxic lymphocytes and complement-requiring cytotoxic antibody in this model as well [121].

### More Recent Observations

One in vitro study of cultures of mouse peritoneal macrophages revealed marked morphological and biochemical changes induced by exposure of cultured macrophages to



PSK. Morphologically, cultures exposed to PSK became more spread out and elongated. Also, protein and DNA synthesis were dramatically increased without an increase in mitogenic activity [48]. Another in vivo study revealed that intra-tumoral injections of PSK increased the number of OKT4+, OKT8+, and IL-2 receptor-positive-cells, causing enhanced antitumor activity by activating the T-cell and reticuloendothelial systems in gastric cancer patients [33]. Another in vivo study of ovarian cancer patients found that PSK increased the OKT 4/8-cell ratio while increasing interleukin-2 (IL-2) production, which was suppressed by cytotoxic chemotherapy [62]. Interestingly, another protein-bound polysaccharide (PSP), also isolated from *Coriolus versicolor*, was found to antagonize the inhibition of IL-2 production by cyclophosphamide from activated T-lymphocytes, restoring T-cell mediated DTH, while increasing phagocytic functions of the reticuloendothelial system and inducing interferon- $\alpha$  and interferon- $\gamma$  [81]. An in vitro study with PSK found that it stimulated TNF-induced cytotoxicity against mouse L-929 fibroblasts, and also stimulated interferon- $\gamma$ -induced differentiation of human myelogenous leukemic U-937 and THP-1 cells, with the highest molecular weight fraction (>200kD) of PSK having the most potent stimulating activity [64]. This ability of PSK to induce differentiation may also be considered a direct anti-tumor effect although it seems to mimic cytokine activity. In a double grafted tumor system, intratumoral injection of PSK as well as oral administration was found to eradicate tumors by increasing numbers of tumor infiltrating lymphocytes(TILs). Significant neutrophil and macrophage chemotactic factor (MCF) activities were detected in the culture media of PSK treated TILs. Furthermore, anti-human IL-8 IgG neutralized neutrophil chemotactic activity, suggesting that PSK acted by inductions of MCF and IL-8-like-factor [11]. Another study suggested that PSK acted to accelerate the transport of T cells from the blood stream to the lymphatics, regulating the distribution of these cells within the body [23]. Also, other studies have looked at the use of specific antibodies [52] and other cytokines [56] in the treatment of malignancies, and have found an additive or synergistic antitumor effect when combined with PSK.

#### Current Understanding

In addition to induction of interferons, MCF, and probably murine IL-8, PSK was found to induce a cytotoxic factor, possibly tumor necrosis factor (TNF), with intravenous administration in normal mice [133]. Although it was previously shown that PSK appeared to induce cytotoxicity by a TNF-independent mechanism, a TNF-dependent mechanism may also exist. This finding may be explained by more recent observations that BRMs like PSK may exert tumoricidal activity by inducing T-cells that recognize them as an antigen and kill tumor cells in an antigen-specific manner, thereby killing tumor targets through TNF-dependent and TNF-independent mechanisms [124]. Recent evidence suggests that PSK does act to potentiate the effects of TNF, and PSK has been shown to stimulate the production of TNF mRNA and gene product as well the secretion of IL-1.

In addition to stimulating the synthesis of various cytokines, PSK has also been shown to increase their activities. PSK was found to increase the TNF- as well as interferon- $\gamma$ -induced differentiation of leukemic cell lines, as well as TNF-cytotoxicity of fibroblastic cell lines, and TNF-stimulated polymorphonuclear cell iodination. These effects may be

partially explained by enhancement of cytokine-receptor binding [95]. Interestingly, fractionation of PSK into four distinct fractions by successive filtration revealed that only the highest molecular weight subfraction with a molecular weight greater than 200 kD (called "F4") had immunologic activities comparable to unfractionated PSK. Also, the unique activities of PSK not observed in other natural or synthetic protein-bound polysaccharides suggest some unique structural features due to the protein portion or sugar linkages that allow such diversity of biological activity.

In summary of known cytokine activity, it has been reported that PSK induces gene expression of several cytokines including TNF- $\alpha$ , IL-1, IL-1R, IL-2, IL-4, IL-6, IL-7 and IL-8, amongst others [18, 61, 165, 169]. These cytokines produced by monocytes, macrophages and other cell types mediate multiple biological effects by direct stimulation of cytotoxic T-cells against tumors, induction of IL-2 receptor expression on T-lymphocytes, and enhancement of antibody production by B-lymphocytes. On the other hand, the use of anti-cytokine antibodies used to experimentally block the actions of specific cytokines--for example the use of anti-TNF antibody [124] as well as antibodies to IFN- $\alpha$ , IFN- $\gamma$ , IL-2, and IL-2 receptors [54]--did not abrogate PSK-induced enhancement of NK cell activity, which suggests that enhanced tumoricidal cytotoxicity is, indeed, caused by more than just manipulation of cytokine production, secretion and binding.

#### New Developments in Cellular and Molecular Biology

The role of BRMs as antigens provoking anti-tumor effects through non-specific stimulation of the immune system has also been supported by one study in which neonatal inoculation of mice with PSK resulted in increased resistance of these mice to the development of metastatic foci following tumor transplantation and to the development of spontaneous tumors following treatment with carcinogen, and induced resistance to challenge with syngeneic tumor cells. These results were not observed in athymic animals, suggesting a definite, though undefined, role of the immune system in these results [86].

More recent studies have shed light on some of the molecular mechanisms responsible for PSK's immunomodulatory actions. In an in vitro comparison of the activities of PSK and IL-2 on NK cells, PSK and IL-2 were found to have equivalent stimulatory activity on NKL cells for induction of cytotoxicity against Daudi tumor cells in a 3-hour  $^{51}\text{Cr}$ -release assay. On further molecular analysis using electrophoretic mobility shift assay, it was suggested that PSK and IL-2 act in different molecular mechanisms to induce NK cell cytotoxicity. PSK appeared to enhance AP-1 and CRE transcription factor binding of stimulated-NK-cell nuclear protein, while IL-2 appeared to enhance AP-1 and SP-1 binding, while inhibiting CRE and NK- $\kappa\text{B}$  binding, and modifying GAS/ISRE, IRF-1 and STAT5 binding [19].

Other very recent studies suggest that another mechanism of PSK's immunomodulatory effect may be through upregulation of the antioxidant enzymes manganese (Mn) superoxide dismutase (SOD) and selenium dependent glutathione peroxidase (SeGSHPx) and non-selenium dependent glutathione peroxidase (non-

SeGSHpx), also called glutathione S-transferase (GST) [125-126, 174]. Although the mechanism of upregulation is uncertain, induction of these enzymes was found to be inhibited by the mRNA synthesis inhibitor actinomycin D, as well as by the protein synthesis inhibitor cycloheximide, suggesting that these enzymes' expression is transcriptionally regulated by PSK, and that induction involved *de novo* protein synthesis. In addition to these cellular effects it is noteworthy that PSK has also been shown to have "SOD-mimicking" activity, which has been demonstrated in a cell-free system in vitro [53]. Further study as to the significance and the exact mechanism of PSK's antioxidant enzyme up-regulation is needed, and may more clearly define its role in immune enhancement and tumor resistance. Another study showed that treatment with PSK can up-regulate inducible nitric oxide synthase (iNOS) gene expression and nitric oxide (NO) production in mouse peritoneal PMNs, which may result in immune system regulation by increase in this important second messenger, though the amount of NO production induced was found to be insufficient for tumor cell killing in vitro [4]. Further studies of the exact molecular effects of PSK on the immune system will help to elucidate and clarify the mechanisms of its immunomodulatory actions, and lead to a better understanding of how it stimulates natural host defense against progression of cancer (figure 2).

### **Anticarcinogenic Effects**

In addition to its immunomodulating anticancer effects in vivo, PSK has also been studied as a chemopreventive agent. The effect of PSK on the process of carcinogenesis has been investigated in chemical carcinogen-induced, radiation-induced, and spontaneously developed animal cancer models, and its potential prophylactic effects have been demonstrated. Of note, PSK has been documented not to interact with or inhibit drug-metabolizing enzymes, and to have no effect on the Ames test, and all experiments have been carried out with attention to the effects of PSK on the metabolism of carcinogens. When Sprague-Dawley rats were injected intravenously with 7,12-dimethyl-benzanthracene (DMBA), oral administration of PSK caused a significant delay in the development of mammary tumors. Also, the formation of tumors of the esophagus, colon, and mammary gland was suppressed by PSK in Donryu rats fed N-n-butyl-N-nitroso urethane (BNUR). Wistar rats injected with 1,2-dimethyl hydrazine (DMH) had prolongation of life and suppression of development of hepatomas when fed PSK, and PSK also inhibited the development of hepatic tumors in Syrian hamsters exposed to the contrast agent thorotrast. In another study, ACI rats fed N-butyl-4-hydroxybutyl-nitrosamine (BBN) developed a decreased number of, and less malignant, bladder tumors when given intraperitoneal injections of PSK. A significant increase in survival and a decrease in serum  $\alpha$ -fetoprotein levels were observed in Wistar rats with 3-methyl-diazobenzene (DAB)-induced hepatomas when fed PSK. Carcinogenesis was also reduced by orally administered PSK in rabbits with N-methyl-N-nitrosourea (MNU)-induced respiratory tract cancers. Intraperitoneal PSK administration was also found to decrease the rate of fibrosarcoma development, and improve survival in C57BL/GN mice and spontaneously hypertensive (SHR) rats exposed to methyl-chloranthene (MCA). Anticarcinogenic effects of PSK were also observed in pregnant rats injected

intravenously with ethylnitrosourea, with fetuses showing suppression of the development of neurogenic tumors [103, 153].

In addition to these and other studies about the preventive effects of PSK against chemical-induced carcinogenesis, the anticarcinogenic effects of PSK have also been demonstrated with regard to radiation-induced cancer. Repeated whole-body irradiation induces lymphoma in the thymus of mice. C57BL/6 mice were exposed to 1.7 Gy whole body irradiation administered once a week for 4 weeks. The group fed 2% PSK showed a significantly inhibited incidence of thymic lymphoma in the tenth month after irradiation. Within one month after irradiation, analysis of thymic lymphocytes revealed probable promotion of differentiation of thymic cells by PSK [69].

The effect of PSK as a chemopreventive agent against the development of spontaneous tumors was also demonstrated in female C3H/He Ouj mice which have a predisposition to develop spontaneous mammary tumors. One year after the initiation of the study, the incidence of tumors and the mean number of tumors (multiplicity) were significantly reduced in the PSK treated group [69].

Immunomodulation is one of the proposed mechanisms of the anti-carcinogenic effects of PSK. Influence on cytokine production and effect, impacts on effector cells, and attenuating properties on immunosuppressive factors may all be of importance in this aspect of PSK's actions. Antiteratogenic effects may also be of great importance in understanding PSK's ability to prevent carcinogenesis. Indeed, PSK has been demonstrated to have strong antiteratogenic effects on chemical- and radiation-induced teratogenesis. Another proposed mechanism PSK's anticarcinogenic effects is the radical trapping effect of PSK. The direct radical trapping effect of PSK in a cell free system in vitro has been observed, confirming that superoxide and hydroxy radicals generated by enzymatic or non-enzymatic reactions were dose-dependently trapped by PSK. Also, as previously stated, PSK seems to have in vivo antioxidant activity by upregulating cellular and mitochondrial antioxidant enzymes. It has been suggested that the upregulation of these enzymes, such as MnSOD, may exert a protective effect against injury to normal cells caused by quinone-type anticancer drugs or irradiation [69].

Finally, another possible mechanism of carcinogenesis prevention by PSK is its ability to suppress chromatid injury and sister chromatid exchange (SCE). Intraperitoneal administration of PSK was found to inhibit the incidence of SCE in the bone marrow of mice exposed to mitomycin C, and this effect has also been documented in mice exposed to cyclophosphamide, nimustine chloride, and x-irradiation [69].

Some direct insight into the anticarcinogenic effects of PSK may be offered by a recent in vitro study showing that certain plant- and mushroom-derived polysaccharides may inhibit reactions considered to be markers for carcinogenesis. This study revealed that a *Coriolus versicolor*-derived polysaccharide preparation (CPS) was found to significantly inhibit 8-hydroxyguanosine (8-OH-dG) formation in cells exposed to the carcinogen benzo[ $\alpha$ ]pyrene (B[ $\alpha$ ]P), and was also found to significantly inhibit superoxide anion formation in phorbol myristic acetate (PMA)-induced HL-60 cells. In

addition, CPS was found to moderately inhibit B[ $\alpha$ ]P-DNA adduct formation, tyrosine kinase activity, and ornithine decarboxylase activity. Interestingly, GST induction in NCTC clone cells was not observed, though levels of other anti-oxidant enzymes known to be induced by PSK were not tested. This study showed that other naturally occurring polysaccharide preparations from *Lentinus edodes*, *Ganoderma lucidum*, and *Aloe barbadensis* Miller, like CPS, also demonstrated significant evidence of anticarcinogenic activity in vitro. The study concluded that these polysaccharides should be considered as potential agents for cancer chemoprevention [65].

The potential use of PSK as a chemopreventive agent for the prevention of second primary cancers, and for the prevention of second malignancies due to chemotherapy and/or radiotherapy is another very intriguing aspect of its use as an adjuvant cancer therapy, and certainly deserves further attention in this regard. Indeed, the apparent diverse antioxidant effects of PSK may provide for an explanation of its numerous biological effects, including prevention of the development of cancer, protection and enhancement of the immune system [80, 173], and improvement in the well-being and general constitution of patients undergoing aggressive cytotoxic cancer therapy [53].

#### **Direct Antitumor/Antimetastatic Effects**

Other than the indirect antitumor effects of cytokine induction and cytotoxic immune enhancement elicited by treatment with this versatile anticancer agent, PSK has also been found to have some very interesting directly antineoplastic effects, including the ability to suppress the progression of cancer by inhibiting the process of metastasis [70].

Early reports suggested some evidence of direct antitumor activity based on observations that PSK could inhibit cancer cell proliferation in vitro. One early study demonstrated that PSK had growth inhibitory effects, and actual mild cytotoxicity for L1210 and P388 tumor cell lines grown in culture [167]. Another early study evaluating the effect of genetic regulation on rat ascites hepatoma tumor cell line AH66, which had previously been shown to be susceptible to the antitumor action of PSK, found, through differential colony hybridization and RNA blot hybridization, that PSK induced two and suppressed one cDNA clone [36].

Although related to cytokine manipulation and immunomodulation, PSK has also been shown to induce differentiation of tumor cells. When mouse macrophage cells from line J774.1 were exposed to PSK, cohabitating human myelogenous leukemic cells were induced to undergo differentiation, which was thought to be due to an increase in TNF, and was also caused by the highest molecular weight subfraction of PSK [132].

PSK also alters prostaglandin metabolism, as previously mentioned [142], and thereby effects platelet aggregation and may also effect attachment of tumor cells to vascular endothelial cells. PSK also inhibits some functions of cytoskeletal proteins such as tubulin, caldesmon, and myosin and may therefore inhibit the extravasation process leading to metastasis [70]. With regard to cell motility, although PSK was found to

enhance the motility of macrophages in vitro, PSK inhibited the motility of tumor cells from Ehrlich, EL-4 lymphoma, and human leukemic cell lines when tested with the capillary method in vitro following incubation with PSK. PSK-treated tumor cells were also found to be less invasive when injected into the abdominal wall of C57BL/6 mice [55]. A similar inhibition of tumor cell mobility through matrigel-coated filters was noted following incubation of murine RL male-1 leukemia cells in PSK, and was proposed to be due to PSK-inhibition of enzymes involved in digestion of basement membranes and extracellular matrices [12]. Similar findings were also observed in a study of aggressively metastatic mouse melanoma cell line B16-BL6, in which PSK was found to suppress in vivo artificial and spontaneous lung metastases, in vitro invasion and chemotaxis, and tumor cell adhesion to, haptotaxis to, and degradation of the basement membrane [90].

Another factor which may be responsible for PSK's direct antitumor activity, and its ability to inhibit metastases may be its ability to inhibit angiogenesis. The anti-angiogenic effects of PSK were observed in the mouse dorsal air sac assay using transplanted MH134 mouse hepatoma cell line. Abundant capillaries rich in the Weibel-Palade, which is a characteristic sign of angiogenesis, were observed in the control group; however, capillary formation was clearly inhibited in mice treated with PSK, and was associated with a decrease in alkaline phosphatase activity of adjacent skin tissues [51].

One of PSK's activities which seems to have a direct antitumor effect while also contributing to its immunomodulating and anticarcinogenic activities, is its ability to induce or mimic SOD. The SOD activity of LLC-WRC-256 (Walker 256 fibrosarcoma) cell lines was found to be less than that of other cell lines in culture. Treatment with PSK was associated with 360% increase in SOD activity, a 256% increase in  $H_2O_2$  concentration, and more than a 50% decrease in cell proliferation rate, with little change in catalase or glutathione peroxidase activity. These findings also suggested that the sensitivity of cancer cells to growth inhibition by PSK might be predetermined based on their pretreatment SOD activity [72]. A similar study of SOD activity in LLC-WRC-256 cell homogenates incubated with PSK revealed a 100% increase in SOD activity of cell homogenate, which was associated with a similar increase in the consumption of nicotinamide adenosine diphosphate (NADPH). This suggests that the mechanism of SOD-mimicking activity of PSK is by collaboration with NADPH as an electron donor in the cytoplasm of cancer cells whose SOD and coupling enzyme activities are significantly lower than in normal cells [73].

Another surprisingly diverse and direct activity of PSK on tumor cells involves the interesting cellular enzymes known as Heat Shock Proteins (HSPs). PSK was found to suppress the expression in human tumor cell lines of HSP47, which is thought to be a collagen-specific molecular chaperone involved in the progression of fibrosis. HSP60, a possible autoantigen in a variety of autoimmune diseases, was also suppressed. No suppression of HSP 72/73 was observed [101]. This activity of PSK may suggest a role for PSK in conditions or states where aberrant HSP expression has been observed, and should probably be considered in more detail when radiotherapy and/or hyperthermia are used as cancer treatments.

The fact that PSK is not a pure compound, and actually has multiple sub-fractions is well documented. Indeed, much of the immunomodulatory activity of PSK has been attributed to highest molecular weight subfraction obtained through successive filtration [133]. Recently, another compound called melanoidin, belonging to a group of melanin-like compounds, was extracted from PSK. Culture of human colon carcinoma cell line HCT-15 and human gastric carcinoma cell line AGS revealed that melanoidin has significant tumor cell proliferation inhibitory effects. Flow cytometric analysis suggested that cell-cycle blocks at S phase and G<sub>2</sub>/M phase were induced by treatment with melanoidin [47]. Further fractionation and separation of PSK, and study of its individual components and their respective actions is obviously needed.

Modern molecular biology techniques have also been used to determine the exact genetic and molecular manipulations induced by PSK, which are considered to be responsible for this drug's direct action on tumor cells. One study of QR-32 tumor cells found that PSK inhibited their *in vitro* and *in vivo* proliferation. Analysis of cytokine mRNA and protein expression revealed that PSK was able to up-regulate MnSOD as well as IFN- $\gamma$ , while maintaining levels of TNF- $\alpha$  and IL-1 $\alpha$ , and decreasing levels of transforming growth factor (TGF)- $\beta$ . These results suggested that PSK suppressed the progression of QR-32 cells by increasing MnSOD via the modulation of inflammatory cytokines, namely by decreasing TGF- $\beta$  and increasing IFN- $\gamma$  [21]. Very similar results were obtained in another recent and similar study using reverse transcriptase polymerase chain reaction (RT-PCR), affirming that this "direct action" on tumor cells was truly indirect—mediated by immunomodulation of cytokines [22].

A study of the effects of PSK on TGF- $\beta$  suggested that PSK formed a complex with TGF- $\beta$ <sub>1</sub> which deactivated the recombinant cytokine. PSK was also suggested to bind with TGF- $\beta$ <sub>2</sub> and platelet-derived growth factor (PDGF), but did not bind with 22 other species of cytokines and growth factors tested. The protein moiety of PSK was found to play an important role in TGF-binding and inactivation [85].

It was also found that treatment of pancreatic carcinoma cell line NOR-P1 and gastric carcinoma cell line MK-1P3 with PSK significantly decreased their invasiveness without significantly affecting their viability, proliferation, or adhesion. PSK was found to inhibit TGF- $\beta$ <sub>1</sub>, matrix metalloproteinase (MMP)-2, and MMP-9 at the mRNA and protein levels, as determined by RT-PCR, gelatin zymography and enzyme-linked immunosorbent assay (ELISA). Western blot analysis revealed that PSK was also found to suppress the expression of urokinase plasminogen activator (uPA) and uPA receptor without changing the expression of plasminogen activator inhibitor-1 (PAI-1). Therefore, PSK's ability to down-regulate TGF- $\beta$ <sub>1</sub>, uPA, MMP-2, and MMP-9 explain its impact in dramatically decreasing tumor cell invasiveness and potential to metastasize [172].

Further inquiry into direct antitumor action of PSK was recently done using human KATO-3 gastric and Colo205 colon carcinoma cell lines. *In vitro* growth of both cell lines was found to be significantly inhibited by incubation with PSK. *In vitro* assessment of invasion was made using a Matrigel invasion chamber and was also found to be

inhibited by PSK. Flow cytometry with monoclonal antibodies to various class I and II human leukocyte antigens (HLAs) revealed that after treatment with PSK, both cell lines demonstrated enhanced expression of various class I and II antigens, suggesting that treatment with PSK may make tumor cells more recognizable to immune surveillance cells, and more susceptible to their cytotoxic effects [42].

A study done in the United States using an extract of *Coriolus versicolor* (with indeterminate amounts of PSK or PSP) also gives some insight into another direct antitumor effect of these versatile polysaccharides. Several prostate cancer cell lines were cultured and treated with an extract of *Coriolus versicolor*, or "Yunzhi" (YZ). The hormone-responsive prostate carcinoma cell line LNCaP was found to display decreased cell growth and decreased secretion of prostate specific antigen (PSA) in response to treatment with YZ; however, the growth inhibition of androgen unresponsive cell lines, JCA-1, PC-3, and DU-145, were much less pronounced. Western blot analysis showed that YZ was able to reduce levels of key cell cycle regulatory proteins, Rb and PCNA, in PC-3 and DU-185 cell lines, respectively. Also, STAT1 and STAT3 transcription factors were found to be increased in JCA-1 cell lines. The conclusion of this study was that *Coriolus versicolor* extract (YZ) should be considered as an effective adjuvant cancer therapy for hormone-responsive prostate cancer, and as a chemopreventive agent to restrict prostate tumorigenic progression from the hormone dependent to the hormone refractory state [38].

### **Results of Selected Recent Clinical Trials**

Decades of clinical trials with PSK have demonstrated its safety and efficacy in cancer patients. To date PSK is considered to be clinically indicated in Japan as an adjuvant cancer therapy for the treatment of stomach, colorectal, lung, breast, esophageal and nasopharynx cancers and may have a role in the adjuvant treatment of leukemia, and several other malignancies [61]. Although further randomized, double blind, placebo controlled trials are still needed to establish the exact indications for the use of PSK as an adjuvant cancer therapy, several landmark studies have already established its efficacy.

#### **Gastric Cancer**

The greatest amount of clinical evidence for the use of PSK is in the role of an adjuvant immunotherapy for the treatment of gastric cancer after curative resection. A recent landmark trial compared the use of PSK combined with standard adjuvant chemotherapy to standard adjuvant chemotherapy alone in patients who had undergone potentially curative resection of biopsy-confirmed primary tumor stage T2 or T3 (Stages I-IV) gastric carcinomas. In this multi-institutional trial, 262 patients were randomly assigned to standard adjuvant treatment alone consisting of chemotherapy with mitomycin-C and 5-FU, or standard adjuvant chemotherapy plus PSK given in a standard dose of 3 grams orally per day for 4 weeks alternating with 5-FU for 10 cycles. With a minimum follow-up time of 5 years, PSK improved both the 5-year disease-free survival rate (70.7% vs. 59.4%,  $p=0.047$ ) and the 5-year overall survival rate (73.0% vs. 60.0%,  $p=0.044$ ) (figure 3a). Both treatment arms were clinically well tolerated and compliance



was good, and it was concluded that the addition of PSK to standard adjuvant chemotherapy for resected gastric cancer was recommended [108]. Interestingly, a review of 872 gastric cancer cases treated with chemoimmunotherapy and subdivided based on levels of carcinoembryonic antigen (CEA), as well as other acute phase reactants (APRs) such as immunosuppressive acidic protein (IAP), acid-soluble glycoproteins,  $\alpha$ 1-antichymotrypsin, and sialic acid, revealed that patients with elevated levels of these APRs benefited most from immunotherapy with PSK [118].

### Colorectal Cancer

PSK has also been used successfully in the adjuvant treatment of colorectal cancer. In one study of 111 patients with Dukes' Stage C (Stages III-IV) colorectal carcinoma, patients were randomized to receive placebo or PSK in step-wise decreasing dosage of 3 grams per day for 2 months, 2g/day for 24 months, and 1g/day thereafter. Treatment with PSK in these high risk patients was found to significantly improve the 8 year overall survival rate (40% vs. 25%,  $p < 0.05$ ) (figure 3b) and the disease free survival rate (25% vs. 8 %,  $p < 0.05$ ) [150].

### Non-small Cell Lung Cancer

Adjuvant therapy of non-small cell lung cancer (NSCLC) is another potential role for PSK. In a study of 185 Stage I-III NSCLC patients treated definitively with external beam radiotherapy, patients with good radiographic response to radiotherapy and good performance status were selected to receive adjuvant post-treatment PSK 3g/day 2 weeks on and 2 weeks off in repeating cycles. The 2-year overall survival rate was dramatically improved in the PSK-treated patients (58% vs. 22%,  $p = 0.000$ ), and the 5 year overall survival was also improved (27% vs. 7%,  $p = 0.000$ ). Despite non-randomization and an obvious patient selection bias, stratification of results revealed that even Stage III patients who received PSK had better outcomes than Stage I and II patients that did not receive PSK, with better 2-year overall survival (44% vs. 32%,  $p = 0.005$ ) and 5-year overall survival rates (22% vs. 16%,  $p = 0.005$ ). Also, when comparison of Stage I-III patients greater than 70 years old was made, the elderly patients who received PSK also had dramatically improved 2-year survival (55% vs. 22%,  $p = 0.007$ ) and 5-year survival (23% vs. 7 %,  $p = 0.007$ ) (figure 3c) over elderly patients not receiving PSK. Stratification of results based on performance status showed that the benefits of PSK were greatest in patients with the best performance status. The authors concluded that immunotherapy following radiotherapy is effective for NSCLC patients, provided their performance status is good and tumor regression in response to radiotherapy is satisfactory [30].

### Breast Cancer

PSK has also been shown to have efficacy in the adjuvant treatment of breast cancer. In a recent study of adjuvant chemotherapy with or without immunotherapy following curative resection of breast cancer with vascular invasion in the primary tumor and/or in the metastatic lymph node, 227 patients were randomized to receive chemotherapy with or without adjuvant immunotherapy. Chemotherapy consisted of 5-FU, cyclophosphamide, mitomycin-c, and prednisolone (FEMP), and immunotherapy was given as PSK or levamisole (LMS). So the three treatment arms for this study included the FEMP alone arm, the FEMP+PSK arm, and the FEMP+LMS arm, with each

treatment carried out at 6-month intervals for 5 years. The trial showed a trend toward improvement in 10-year overall survival with the FEMP+PSK group having the best prognosis, the FEMP+LMS group having the next best prognosis, and the FEMP alone group having the worst prognosis; however, the differences did not reach statistical significance (81.1% vs. 76.9% vs. 64.6%,  $p=0.1686$ ) (figure 3d). There was also a trend toward disease free survival being best in the FEMP+PSK arm, intermediate in the FEMP+LMS arm, and worst in the FEMP alone arm, but also did not reach statistical significance (74.1% vs. 70.7% vs. 64.6%,  $p=0.3141$ ). The conclusion of this study was that immunotherapy with PSK improves the prognosis of operable breast cancer patients with vascular invasion [43]. Though the benefits of PSK in this breast cancer study were not statistically significant, further stratification of the patients who took PSK by dividing them into HLA B40 antigen-positive or -negative revealed that HLA B40 antigen-positive patients who had taken PSK had almost double the overall survival of HLA B40 antigen-negative patients (100% vs.  $\approx 50\%$ ,  $p<0.05$ ) (figure 3e). The conclusion of this subset analysis was that breast cancer patients who are HLA B40-positive might derive great benefit from adjuvant immunotherapy with PSK, while HLA B40-negative breast cancer patients may derive little or no benefit [170].

#### Esophageal Cancer

A recent multi-center randomized study of 158 esophageal cancer patients who underwent radical esophageal resection followed by radiotherapy had 4 treatment arms consisting of adjuvant chemotherapy with or without PSK, or no chemotherapy with or without PSK. Stratification of patients based on levels of the immunosuppressive serum tumor markers  $\alpha$ -1-anti-chymotrypsin and sialic acid levels revealed that patients with elevated serum levels of  $\alpha$ -1-anti-chymotrypsin had much better 5-year overall survival if given PSK (55% vs. 26%,  $p<0.008$ ). Likewise, patients with elevated sialic acid levels had greater 5-year overall survival if they received adjuvant PSK (58% vs. 31%,  $p<0.07$ ) (figure 3f). The conclusion of this study was that adjuvant immunotherapy with PSK improves the survival of esophageal cancer patients, especially those with elevated levels of one or both of these tumor markers [119].

#### Nasopharynx Cancer

A small randomized clinical study of the use of immunotherapy with PSK following standard radiotherapy with or without chemotherapy was conducted on 38 patients with nasopharyngeal carcinoma. Although PSK demonstrated no improvement in local control, there was an improvement in the rate of distant metastasis with PSK (14% vs. 35%) and in the median survival (35 months vs. 25 months,  $p=0.043$ ) and 5-year survival (28% vs. 15%,  $p=0.043$ ) rates (figure 3g). The conclusion of this study was that PSK deserved careful consideration as an important immunotherapeutic agent in the management of nasopharyngeal carcinoma [20].

#### Leukemia

An early prospective randomized cooperative trial of chemoimmunotherapy for acute myelogenous leukemia (AML) using PSK was conducted. Following complete remission with induction chemotherapy and consolidation therapy, 73 patients were randomized to

receive maintenance chemotherapy with or without PSK. Though no statistically significant benefit in duration of remission or survival was demonstrated, of the patients that maintained remissions for more than 270 days, there was an indication that PSK prolonged the 50% remission period by 418 days (885 days vs. 467 days,  $p=.105$ ) [122].

Another more recent study of pediatric acute lymphoblastic leukemia (ALL) assessed the use of various biological response modifiers (BRMs) in patients in complete remission following treatment with chemotherapy. Although BRMs did seem to be effective in preventing relapse, no statistically significant benefit was seen in patients using PSK, though PSK was used safely in all the pediatric patients studied [59].

### **Potential Use as a Normal Tissue Radioprotector and Tumor Radiosensitizer**

The use of PSK in conjunction with radiotherapy in treatment of cancer offers a new innovative strategy that may minimize complications of radiotherapy and has the potential to enhance cure rates. When PSK was administered after definitive radiotherapy to C3H/He mice transplanted with MM46 tumors, tumor growth was decreased and 60-day survival was increased significantly [97]. Also, when various polyglycans including PSK were administered to C3H/HeN mice after cobalt-60 irradiation, colony formation assessed by endogenous spleen colony assay was dramatically enhanced [128]. The use of immunotherapy with radiation is also emphasized in some studies, demonstrating enhanced lymphocyte infiltration into tumors, greater local control and survival rates, and decreased risk of distant metastases when radiation and PSK are used in conjunction [37, 94]. One in vivo study examining the radioprotective effects of PSK revealed that PSK could reduce or prevent X-irradiation-induced congenital malformations caused by radiation exposure in utero, and antiteratogenic effects and suppression of early fetal death secondary to radiation exposure were observed due to treatment with PSK [84]. Two other studies looked at the effects of PSK and an extract of *Ganoderma lucidum* (Gl) on protection against gamma irradiation in mature mice. The first study revealed that both polysaccharide preparations were effective in enhancing the recovery of cellular immunocompetence from gamma-irradiation as assessed by splenic weights,  $^3\text{H}$ -thymidine incorporation into mitogen-stimulated spleen cells, and leukocyte counts [8]. The other study confirmed this finding through comparison of thymic weights and assessment of restoration of T-cell subsets [9].

In an attempt to reduce mucositis in patients undergoing head and neck irradiation, treatment with filgrastim, or recombinant methionyl human granulocyte colony-stimulating factor (r-metHuG-CSF) was found to moderately decrease the severity of radiation-induced mucositis [135]. In addition the radioprotector amifostine has been used with moderate success in the prevention of radiation-induced oral and oropharyngeal mucositis [6]. Furthermore, the increased radiosensitivity of AIDS patients implies that the immune system plays an important part in the protection of normal cells from the effects of radiation [14, 39, 127, 163]. Finally, PSK's abilities to mimic and induce expression of MnSOD may not only increase the radiosensitivity of tumors [162], but may also have a protective effect on normal tissues [13].

Taken together these findings make it likely that PSK and other protein-bound polysaccharides, with their immunostimulating and multifaceted antioxidant activity, may play a potential role as normal tissue radioprotectors when used in combination with radiotherapy in the treatment of cancer. Further investigation of the radioprotective and radiopotentiating aspects of protein-bound polysaccharides is warranted.

### **Potential Use as a Chemotherapy Protector**

The use of PSK as an agent to protect against the deleterious effects of cytotoxic chemotherapy has also been suggested. As alluded to in the discussion of the anticarcinogenic effects of irradiation, PSK and Lentinan were found to inhibit the rate of sister chromatid exchange (SCE) induced by the quinone chemotherapy agent mitomycin-C. This inhibition of chromosomal damage may have an antiteratogenic and anticarcinogenic effect, and may also protect normal somatic cells from irreparable genetic alteration [28]. Further antiteratogenic activity of PSK was demonstrated in a study of PSK and levamisole, which showed that both compounds suppressed 5-azacytidine-induced digital malformations in the rat, though the mechanism of antiteratogenicity is unknown [79].

Though PSK was found to have no effect on colony formation stimulated by erythropoietin and medium conditioned by phytohemagglutinin-stimulated leukocytes, medium conditioned by PSK-stimulated leukocytes significantly stimulated formation of various types of colonies including erythroid bursts, granulocyte, macrophage, eosinophil, megakaryocyte, and mixed hemopoietic colonies. Due to these findings it was speculated that administration of the optimal dose of PSK could reduce the hematological suppression of cytotoxic chemotherapy [151]. Indeed, animal experiments showed that administration of PSK could decrease myelosuppression following chemotherapy. Also, granulocyte-colony stimulating factor (G-CSF), granulocyte/macrophage-colony stimulating factor (GM-CSF), interleukin-3 (IL-3), and other cytokines are now being employed clinically and have been recognized to accelerate the recovery from chemotherapy-induced granulocytopenia. One study examined the use of PSK with G-CSF, GM-CSF, or IL-3, and found that combined administration of PSK with these cytokines increased the hematological recovery in myelosuppressed mice [74]. Other researchers observed that PSK was slightly weaker than G-CSF in preventing chemotherapy-induced leukopenia, but that PSK was a better enhancer of beneficial chemotherapy effect. Also, serum levels of immunosuppressive acidic protein (IAP) and interleukin-2 receptor (sIL-2R) were found to be good indices for predicting response to chemotherapy and immunotherapy [58].

As mentioned previously, PSK may also act to relieve the oxidative stress on cancer patients, that is thought to be partially due to local ischemia and hypoxia in tumors [53]. Many chemotherapy agents are also generators of reactive oxygen species, increasing the oxidative stress on cancer patients. One study found that PSK had the ability to suppress the increase in lipid peroxide and the decrease in SOD activity in normal rat kidney

fibroblast cell line NRK-49F induced by treatment with cisplatin. On the other hand, PSK augmented the formation of lipid peroxide and the decrease in SOD activity in rat hepatoma cell line H4-II-E and human ovarian cancer cells induced by cisplatin. This study concluded that PSK may have a selectively chemoprotective effect on normal cells, along with a selectively chemosensitizing effect on cancer cells [71].

### **Potential Use as a Post-Surgical Immunosuppression Inhibitor**

Reduction of the quantity and activity of immunosuppressive substances caused by cancer is one of the characteristics of PSK. An increase of nonspecific immunosuppressive substances in the body fluid of cancer-bearing hosts and abnormal augmentation of suppressor cells is involved in the mechanism of cancer-induced immunosuppression [69, 87-89, 115, 153]. Also, immunosuppression caused by surgical stress may adversely effect the prognosis of patients by interfering with normal immune defense against residual cancer cells or tumors remaining after surgery. A study of cancer patients undergoing the surgical stress of esophagectomy or gastrectomy found that pretreatment of patients with PSK resulted in decreased post-surgical immunosuppression as determined by levels of subsets of T-cells including CD4+2H4- (helper T-), CD8+CD11- (cytotoxic T-), and CD4+2H4+ (suppressor/inducer T-) cells. It has been inferred that PSK may improve the prognosis of cancer patients undergoing radical surgery by inhibiting immunosuppression secondary to surgical stress [116, 148].

### **Antimicrobial and Antiviral Effects**

In addition to its multiple anticancer properties, PSK has also been reported to have in vitro and in vivo antiviral effects. PSK was initially found to protect mice from ectromelia and cytomegalovirus infection in vivo [153]. PSK was also found to block the cytopathic effect of human immunodeficiency virus (HIV) in vitro, such as multinucleated giant cell formation and HIV-specific antigen expression in MT-4 and MOLT-4 cells. Pretreatment of these cell lines with PSK was thought to interfere with the early stages of HIV infection by modifying the viral receptor [146]. Cell-free viral infection and the fusion reaction induced by cell-to-cell infection by HIV-1, HIV-2, and human T-cell lymphotropic virus (HTLV)-I was also inhibited by PSK [147]. PSK was also found to non-competitively inhibit reverse transcriptase in vitro, contributing to its observed antiviral effect [35]. In accordance with PSK's proposed chemoprotective properties, PSK was found to protect mice from immunosuppression induced by treatment with cyclophosphamide as determined by inoculation with influenza virus [159]. PSK has also been demonstrated to inactivate strains of herpes simplex virus (HSV)-types 1 and 2 in vitro, by inhibiting synthesis of viral proteins [100].

PSK has also been shown to have antimicrobial effects. PSK exhibited in vivo anti-candidal activity in mice, as well in vitro anti-toxoplasmosis activity [110]. PSK was found to partially protect immunosuppressed tumor-bearing mice from infection with *Candida albicans*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Escherichia coli*,

and *Streptococcus pneumoniae* [3, 153]. PSK was also shown to have a preventive effect on the abnormal conditions of the intestinal flora of mice inoculated with tumor cells or exposed to chemotherapy [134].

### **Summary**

The various basic science and clinical research that has been conducted on PSK and other protein-bound polysaccharides confirms that these are potent BRMs with diverse biological effects. Further clarification of the active components of these preparations, as well as further elucidation of their respective mechanisms of action may provide an innovative modality to be used in conjunction with other conventional therapies in the treatment of cancer. Further studies should be conducted to determine the exact cellular and molecular mechanisms responsible for their many biological effects, with close correlation of structure and function. These potent immunoceuticals may offer additional hope to cancer patients with their unique functions including immunostimulating, anticarcinogenic, antimetastatic, antioxidant, antimicrobial, antiviral, and chemo- and radio-protective activities.

### **Figure Legends**

Figure 1: Proposed molecular structure of protein-bound polysaccharide (PSK) with peptide core and polysaccharide branches. (Modified from Kidd, 2000 [61], with permission.)

Figure 2: Proposed immunomodulatory and anticancer pathways of protein-bound polysaccharide PSK. (Modified from Kidd, 2000 [61], with permission.)

Figure 3(a-g): Improvement in overall survival in selected recent clinical trials using adjuvant immunotherapy with PSK. (Modified from Kidd, 2000 [61], with permission.)

## References

1. Agha-Mohammadi S, Lotze MT. (2000) Immunomodulation of cancer: potential use of selectively replicating agents. *Journal of Clinical Investigation* 105:1173-1176
2. Ando T, Mastuda Y, Matsunaga K, Fujii T, Yoshikumi C, Nomoto K. (1984) Effect of PSK on the recovery of macrophage function in tumor-bearing mice. *Japanese Journal of Cancer & Chemotherapy*. 11:827-834
3. Ando T, Motokawa I, Sakurai K, Ohmura Y, Matsunaga K, Fujii T, Yoshikumi C, Taguchi T. (1988) Effects of PSK on resistance to bacterial infection in splenectomized mice. *Oncology* 45:224-229
4. Asai K, Kato H, Hirose K, Akaogi K, Kimura S, Mukai S, Inoue M, Yamamura Y, Sano H, Sugino S, Yoshikawa T, Kondo M. (2000) PSK and OK-432-induced immunomodulation of inducible nitric oxide (NO) synthase gene expression in mouse peritoneal polymorphonuclear leukocytes and NO-mediated cytotoxicity. *Immunopharmacology & Immunotoxicology* 22:221-235
5. Asaishi K, Okazaki M, Hayasaka H. (1988) Local treatment of locally recurrent breast cancers. *Japanese Journal of Cancer & Chemotherapy*. 15:1325-1330
6. Brizel DM, Wasserman TH, Henke M, Strnad V, Rudat V, Monnier A, Eschwege F, Zhang J, Russell L, Oster W, Sauer R. (2000) Phase III randomized trial of amifostine as a radioprotector in head and neck cancer. *Journal of Clinical Oncology* 18:3339-3345
7. Chihara G, Maeda Y, Hamuro J, Sasaki T, Fukuoka F. (1969) Inhibition of mouse sarcoma 180 by polysaccharides from *Lentinus edodes* (Berk.) sing. *Nature* 222:687-688
8. Chen WC, Hau DM, Lee SS. (1995) Effects of *Ganoderma lucidum* and krestin on cellular immunocompetence in gamma-ray-irradiated mice. *American Journal of Chinese Medicine* 23:71-80
9. Chen WC, Hau DM, Wang CC, Lin IH, Lee SS. (1995) Effects of *Ganoderma lucidum* and krestin on subset T-cell in spleen of gamma-irradiated mice. *American Journal of Chinese Medicine* 23:289-298
10. Chung CH, Go P, Chang KH. (1987) PSK immunotherapy in cancer patients—a preliminary report. *Chinese Journal of Microbiology & Immunology*. 20:210-216
11. Ebina T, Murata K. (1990) Antitumor effect of PSK at a distant site: inductions of interleukin-8-like factor and macrophage chemotactic factor in murine tumor. *Japanese Journal of Cancer Research* 81:1307-1313
12. Ebina T, Murata K. (1994) Antitumor effect of intratumoral administration of a *Coriolus* preparation, PSK: inhibition of tumor invasion in vitro. *Japanese Journal of Cancer & Chemotherapy* 21:2241-2243
13. Epperly MW, Kagan VE, Sikora CA. (2001) Manganese superoxide dismutase-plasmid/liposome (MnSOD-PL) administration protects mice from esophagitis associated with fractionated radiation. *International Journal of Cancer* 96:221-231
14. Formenti SC, Chak L, Gill P, Buess EM, Hill CK. (1995) Increased radiosensitivity of normal tissue fibroblasts in patients with acquired immunodeficiency syndrome (AIDS) and with Kaposi's sarcoma. *International Journal of Radiation Biology* 68:411-412
15. Fujita H, Ogawa K, Ikuzawa M, Muto S, Matsuki M, Nakajima S, Shimamura M, Togawa M, Yoshikumi C, Kawai Y. (1986) Effects of krestin (PSK) on drug-



- metabolizing enzymes with special reference to the activation of FT-207. Japanese Journal of Cancer & Chemotherapy 13:2653-2657
16. Fujita H, Ogawa K, Ikuzawa M, Muto S, Matsuki M, Nakajima S, Shimamura M, Togawa M, Yoshikumi C, Kawai Y. (1988) Effect of PSK, a protein-bound polysaccharide from *Coriolus versicolor*, on drug-metabolizing enzymes in sarcoma-180 bearing and normal mice. International Journal of Immunopharmacology 10:445-450
  17. Fukuo Y, Terashi A, Hayashi Y, Yano Y, Atarashi J. (1985) A clinical trial of cis-platinum (II) in combination with PSK and FT-207 in advanced stomach cancer. Japanese Journal of Cancer & Chemotherapy. 12:960-965
  18. Fung L, Ooi V, Chang S. (1996) Induction in the mouse of gene expression of immunomodulating cytokines by mushroom polysaccharide-protein complexes. Life Sciences 58:1795-1803
  19. Garcia-Lora A, Pedrinaci S, Garrido F. (2001) Protein-bound polysaccharide K and interleukin-2 regulate different nuclear transcription factors in the NKL human natural killer cell line. Cancer Immunology, Immunotherapy 50:191-198
  20. Go P, Chung CH. (1989) Adjuvant PSK immunotherapy in patients with carcinoma of the nasopharynx. Journal of International Medical Research. 17:141-149
  21. Habelhah H, Okada F, Nakai K, Choi SK, Hamada J, Kobayashi M, Hosokawa M. (1998) Polysaccharide K induces Mn superoxide dismutase (Mn-SOD) in tumor tissues and inhibits malignant progression of QR-32 tumor cells: possible roles of interferon alpha, tumor necrosis factor alpha and transforming growth factor beta in Mn-SOD induction by polysaccharide K. Cancer Immunology, Immunotherapy 46:338-344
  22. Habellah H. (1998) Induction of manganese superoxide dismutase by an immunopotentiator as a mechanism of inhibiting malignant progression of murine tumor cells. Hokkaido Journal of Medical Science 73:519-529
  23. Hanaue H, Tokuda Y, Fujimoto T, Mitzutani K, Okumura A, Kamijoh A, Ogoshi K, Makuuchi H, Nakasaki H, Tajima T. (1990) The effect of protein-bound polysaccharide (PSK) on lymphocyte subsets of peripheral venous blood and thoracic duct lymph. Journal of Japan Society for Cancer Therapy 25:1410-1418
  24. Hara M, Nakagami Y, Hiraoka Y, Lin T. (1985) A case of bilateral renal cell carcinoma. Acta Urologica Japonica. 31:1787-1791
  25. Hara Y, Okazaki M, Higashihara H, Hara H. (1988) Disappearance of lung metastases from hepatocellular carcinoma following PSK administration. Japanese Journal of Clinical Radiology. 33:731-734
  26. Haranaka K, Satomi N, Sakurai A, Haranaka R, Okada N, Kobayashi M. (1985) Antitumor activities and tumor necrosis factor producibility of traditional Chinese medicines and crude drugs. Cancer Immunology, Immunotherapy 20:1-5
  27. Haranaka K, Satomi N, Sakurai A, Haranaka R. (1984) Role of first stimulating agents in the production of tumor necrosis factor. Cancer Immunology, Immunotherapy 18:87-90
  28. Hasegawa J, Hosokawa M, Okada F, Kobayashi H. (1989) Inhibition of mitomycin-C-induced sister-chromatid exchanges in mouse bone marrow cells by the immunopotentiators krestin and lentinan. Mutation Research 226:9-12

29. Hattori T, Niimoto M, Koh T, Nakano A, Oride M, Takiyama W, Nishimawari K. (1979) Post-operative long-term adjuvant immunochemotherapy with mitomycin-C, PSK, and FT-207 in gastric cancer patients. *Japanese Journal of Surgery*. 9:110-117
30. Hayakawa K, Mitsunashi N, Saito Y, Takahashi M, Katano S, Shiojima K, Furuta M, Niibe H. (1993) Effect of krestin (PSK) as adjuvant treatment on the prognosis after radical radiotherapy in patients with non-small cell lung cancer. *Anticancer Research* 13:1815-1820
31. Hayashi Y. (1988) Enhancing effect of irradiation on carcinoma of the uterine cervix by administering the protein-bound polysaccharide kureha (PSK)—quantitative nuclear DNA analysis following irradiation. *Acta Obstetrica et Gynaecologica Japonica* 40:179-186
32. Hayashi Y, Komada S, Maruyama Y, Hirao Y, Okajima E. (1987) Primary transitional cell carcinoma of the male urethra: report of a case. *Acta Urologica Japonica*. 33:428-432
33. Hiramatsu S. (1987) Immunohistochemical study of spleens in gastric cancer. *Journal of Japan Surgical Society* 88:1591-1603
34. Hiramatsu Y, Fukui H, Sekiba K, Taguchi K. (1983) Case report of a large endometrial stromal sarcoma. *Japanese Journal of Cancer Clinics*. 29:A-24, 375-380
35. Hirose K, Hakozaiki M, Kakuchi J, Matsunaga K, Yoshikumi C, Takahashi M, Tochikura TS, Yamamoto N. (1987) A biological response modifier, PSK, inhibits reverse transcriptase in vitro. *Biochemical & Biophysical Research Communications* 149:562-567
36. Hirose K, Hakozaiki M, Matsunaga K, Yoshikumi C, Hotta T, Yanagisawa M, Yamamoto M, Endo H. (1985) Cloning of sequences induced and suppressed by administration of PSK, antitumor protein-bound polysaccharide. *Biochemical and Biophysical Research Communications* 126:884-892
37. Hirota S, Ogawa Y, Seguchi H. (1987) Histological analysis of lymphocyte subsets infiltrated into mouse tumor tissue exposed to local irradiation. *Oncology* 44:312-318
38. Hsieh T, Wu J. (2001) Cell growth and gene modulatory activities of Yunzhi (Windsor Wunxi) from mushroom *Trametes versicolor* in androgen-dependent and androgen-insensitive human prostate cancer cells. *International Journal of Oncology* 18:81-88
39. Hughes-Davies L, Young T, Spittle M. (1991) Radiosensitivity in AIDS patients. *Lancet* 337:918-919]
40. Ichihashi H, Kondo T, Nakazato H. (1987) Clinical results of a randomized controlled trial on the effect of adjuvant immunochemotherapy using esquinon and krestin in patients with curatively resected gastric cancer—7-year survival—Cooperative Study Group for Cancer Immunochemotherapy, Tokai Gastrointestinal Oncology Group. *Japanese Journal of Cancer & Chemotherapy*. 14:2758-2766
41. Ichihashi H, Kondo T, Yamauchi M, Isomatsu T, Nakamura T, Sakabe T, Nakajima T, Ikenaga T, Shiratori T, Taguchi T. (1985) A cooperative study of alternating immunochemotherapy with futraful and PSK (second report)—3-year survival rate. *Japanese Journal of Cancer & Chemotherapy*. 12:331-336
42. Iguchi C, Nio Y, Takeda H, Yamasawa K, Hirahara N, Toga T, Itakura M, Tamura K. (2001) Plant polysaccharide PSK: cytostatic effects on growth and invasion;

- modulating effect on the expression of HLA and adhesion molecules on human gastric and colonic tumor cell surface. *Anticancer Research* 21:1007-1014
43. Iino Y, Yokoe T, Maemura M, Horiguchi J, Takei H, Ohwada S, Morishita Y. (1995) Immunochemotherapies versus chemotherapy as adjuvant treatment after curative resection of operable breast cancer. *Anticancer Research* 15:2907-2912
  44. Ikeda T, Sakai T, Suito T, Kosaki G. (1986) Evaluation of postoperative immunochemotherapy in lung cancer. *Japanese Journal of Cancer & Chemotherapy* 13:1044-1049
  45. Ikuzawa M, Matsunaga K, Nishiyama S, Nakajima S, Kobayashi Y, Andoh T, Kobayashi A, Ohhara M, Ohmura Y, Wada T. (1988) Fate and distribution of an antitumor protein-bound polysaccharide PSK (krestin). *International Journal of Immunopharmacology* 10:415-423
  46. Isono K, Sato H, Uematsu S, Sato H, Koike Y. (1979) Radiotherapy, bleomycin, and immunotherapy (PSK, BCG-CWS) in esophageal neoplasms. *Journal of the Japanese Association for Thoracic Surgery*. 27:496-498
  47. Kamei H, Hashimoto Y, Koide T, Kojima T, Hasegawa M, Umeda T. (1997) Direct tumor growth suppressive effect of melanoidin extracted from immunomodulator PSK. *Cancer Biotherapy & Radiopharmaceuticals* 12:341-344
  48. Kamisato J, Nowakowski M. (1988) Morphological and biochemical alterations of macrophages produced by a glycan, PSK. *Immunopharmacology* 16:89-96
  49. Kanabe S, Tamakuma S, Mimura K, Hiraide H, Mochizuki H, Tamaki K, Kurokawa T, Kadota T, Hatsuse K, Kawano M. (1985) Comparison of immunochemotherapy and chemotherapy of Stage IV gastric carcinoma. *Japanese Journal of Cancer Clinics*. 31:1805-1809
  50. Kano T, Kumashiro R, Masuda H, Tamada R, Inokuchi K. (1983) Late results of postoperative long-term cancer chemotherapy for the gastric cancer patients subjected to curative resection. *Japanese Journal of Surgery* 13:112-116
  51. Kanoh T, Matsunaga K, Saito K, Fujii T. (1994) Suppression of in vivo tumor-induced angiogenesis by the protein-bound polysaccharide PSK. *In Vivo* 8:247-250
  52. Kanoh T, Saito K, Matsunaga K, Oguchi Y, Taniguchi N, Endoh H, Yoshimura M, Fujii T. (1994) Enhancement of the antitumor effect by the concurrent use of a monoclonal antibody and the protein-bound polysaccharide PSK in mice bearing a human cancer cell line. *In Vivo* 8:241-245
  53. Kariya K, Nakamura K, Nomoto K, Matam S, Saigenji K. (1992) Mimicking of superoxide dismutase activity by protein-bound polysaccharide of *Coriolus versicolor* QUEL, and oxidative stress relief for cancer patients. *Molecular Biotherapy* 4:40-46
  54. Kariya Y, Inoue N, Kihara T, Okamoto N, Sugie K, Mori T, Uchida A. (1992) Activation of human natural killer cells by the protein-bound polysaccharide PSK independently of interferon and interleukin-2. *Immunology Letters* 31:241-246
  55. Katano M, Yamamoto H, Torisu M. (1987) A suppressive effect of PSK, a protein-bound polysaccharide preparation, on tumor growth: a new effect of PSK on cell motility. *Japanese Journal of Cancer & Chemotherapy* 14:2321-2326
  56. Kato H, Inoue M, Yamamura Y, Kawahito Y, Mukai S, Sugino S, Kondo M. (1992) Effect of recombinant human granulocyte colony-stimulating factor (rhG-CSF) on cytotoxicity of PSK-induced peritoneal polymorphonuclear leukocytes (PMNs). *Biotherapy* 5:177-186

57. Kato H, Yokoe N, Takemura S, Yoshikawa T, Furukawa Y, Kondo M. (1983) Effect of a protein-bound polysaccharide PSK on the complement system. *Research in Experimental Medicine*. 182:85-94
58. Kato R, Takenoshita S, Shimizu Y, Tanaka S, Yajima Y, Nagamachi Y. (1997) Changes in serum soluble IL-2 receptors (sIL-2R) and immunosuppressive acidic protein (IAP) associated with chemotherapy for lung cancer. *Anticancer Research* 17:3787-3792
59. Kawa K, Konishi S, Tsujino G, Mabuchi S. (1991) Effects of biological response modifiers on childhood ALL being in remission after chemotherapy. *Biomedicine & Pharmacotherapy* 45:113-116
60. Kawano K, Tada I, Suzuki K, Nakashima K, Yoshida T, Mitarai Y, Kim YI, Kobayashi M. (1989) A case of recurrent hepatocellular carcinoma successfully treated by arterial chemoembolization in combination with local hyperthermia. *Japanese Journal of Cancer & Chemotherapy*. 16:2957-2960
61. Kidd P. (2000) The use of mushroom glucans and proteoglycans in cancer treatment. *Alternative Medicine Review* 5:4-27
62. Kikuchi Y, Kizawa I, Oomori K, Iwano I, Kita T, Miyauchi M, Kato K. (1987) Effects of cimetidine and PSK on interleukin-2 production by PBL in patients with advanced ovarian carcinoma during the course of chemotherapy. *Acta Obstetrica et Gynaecologica Japonica*. 39:1987-1992
63. Kikuchi Y, Kizawa I, Oomori K, Iwano I, Kita T, Kato K. (1988) Effects of PSK on interleukin-2 production by peripheral lymphocytes of patients with advanced ovarian carcinoma during chemotherapy. *Japanese Journal of Cancer Research*. 79:125-130
64. Kim F, Sakagami H, Tanuma S, Konno K. (1990) Stimulation of interferon-gamma-induced human myelogenous leukemic cell differentiation by high molecular weight PSK subfraction. *Anticancer Research* 10:55-58
65. Kim H, Kacew S, Lee B. (1999) In vitro chemopreventive effects of plant polysaccharides (*Aloe barbadensis* Miller, *Lentinus edodes*, *Ganoderma lucidum* and *Coriolus versicolor*). *Carcinogenesis* 20:1637-1640
66. Kitahara M, Katakura R, Mashiyama S, Niizuma H, Yoshimoto T, Suzuki J, Mori T, Wada T. (1987) Results in oligodendroglioma: postoperative radiotherapy combined with chemotherapy. [Japanese] *Neurological Surgery*. 15:397-403
67. Kitahara M, Katakura R, Shingai J, Niizuma H, Yoshimoto T, Suzuki J, Mori T, Wada T. (1987) Results of supratentorial low-grade astrocytoma—effects of combined radiochemotherapy. [Japanese] *Neurological Surgery*. 15:597-604
68. Kitani H, Tsuru S, Oguchi M, Watanabe M, Zinnaka Y. (1984) Effect of PSK on interferon production in tumor-bearing mice. *Journal of Clinical & Laboratory Immunology*. 15:211-214
69. Kobayashi H, Matsunaga K, Masahiko F. (1993) PSK as a chemopreventive agent. *Cancer Epidemiology, Biomarkers & Prevention* 2:271-276
70. Kobayashi H, Matsunaga K, Oguchi Y. (1995) Antimetastatic effects of PSK (krestin), a protein-bound polysaccharide obtained from basidiomycetes: an overview. *Cancer Epidemiology, Biomarkers & Prevention* 4:275-281
71. Kobayashi Y, Kariya K, Saigenji K, Nakamura K. (1994) Enhancement of anti-cancer activity of cisdiaminedichloroplatinum by the protein-bound polysaccharide of *Coriolus versicolor* QUEL (PSK) in vitro. *Cancer Biotherapy* 9:351-358

72. Kobayashi Y, Kariya K, Saigenji K, Nakamura K. (1994) Suppression of cancer cell growth in vitro by the protein-bound polysaccharide of *Coriolus versicolor* QUEL (PSK) with SOD mimicking activity. *Cancer Biotherapy* 9:63-69
73. Kobayashi Y, Kariya K, Saigenji K, Nakamura K. (1994) Suppressive effects on cancer cell proliferation of the enhancement of superoxide dismutase (SOD) activity associated with the protein-bound polysaccharide of *Coriolus versicolor* QUEL. *Cancer Biotherapy* 9:171-178
74. Kohgo Y, Hirayama Y, Sakamaki S, (1994) Improved recovery of myelosuppression following chemotherapy in mice by combined administration of PSK and various cytokines. *Acta Haematologica* 92:130-135
75. Kojima E, Mitsuno T, Osawa T. (1986) Identification of a mouse serum protein increased by administration of an antitumor polysaccharide, PSK, as a variant of mouse transferrin and some of its biological activities. *Journal of Pharmacobio-Dynamics* 9:174-181
76. Kojima J, Kamata T, Monna T. (1987) Evaluation of immunochemotherapy in patients with primary liver cancer. *Osaka Research Society for Liver, Gallbladder and Pancreas. Japanese Journal of Cancer & Chemotherapy.* 14:179-187
77. Komaki H, Onizuka Y, Mihara K, Seita M, Maeda M, Nakamura S. (1988) Successful treatment with tegafur and PSK in a patient with gallbladder cancer. *Japanese Journal of Cancer & Chemotherapy.* 15:2801-2804
78. Kondo N, Hongo H, Nakayasu K, Shinagawa H, Ishuzu K, Misumi K, Kuroda Y, Kurata S, Ohfuji K. (1987) A case report of successful immunochemotherapy of metastatic renal cell carcinoma using UFT and PSK. *Japanese Journal of Cancer & Chemotherapy.* 14:2568-2570
79. Kurishita A. (1990) Suppressive effects of two biological response modifiers, krestin and levamisole, on 5-azacytidine-induced digital defects in rats. *Teratogenesis, Carcinogenesis, & Mutagenesis* 10:409-415
80. Li J, Zhou M, Chen Y. (1993) Reversal of inhibition of reactive oxygen species on respiratory burst of macrophages by polysaccharide from *Coriolus versicolor*. *International Journal of Immunopharmacology* 15:429-433
81. Li X, Wang J, Zhu P, Liu L, Ge J, Yang S. (1990) Immune enhancement of a polysaccharide peptide isolated from *Coriolus versicolor*. *Acta Pharmacologica Sinica* 11:542-545
82. Maehara Y, Moriguchi S, Sakaguchi Y, (1990) Adjuvant chemotherapy enhances long-term survival of patients with advanced gastric cancer following curative resection. *Journal of Surgical Oncology.* 45:169-172
83. Malone MH, Tyler VE, Brady LR. (1967) Hippocratic screening of sixty-six species of higher fungi. *Lloydia* 30:250-257
84. Matsui H, Setogawa T, Naora H, Tanaka O. (1995) The effects of PSK, a biological response modifier, on congenital ocular abnormalities induced by X-irradiation. *Histology & Histopathology* 10:47-54
85. Matsunaga K, Hosokawa A, Oohara M, Sugita N, Harada M, Nomoto K. (1998) Direct action of a protein-bound polysaccharide, PSK, on transforming growth factor-beta. *Immunopharmacology* 40:219-230
86. Matsunaga K, Iijima H, Kobayashi H. (2000) Neonatal inoculation with the protein-bound polysaccharide PSK increases resistance of adult animals to challenge with

- syngeneic tumor cells and reduces azoxymethane-induced precancerous lesions in the colon. *Cancer Epidemiology, Biomarkers & Prevention* 9:1313-1322
87. Matsunaga K, Morita I, Oguchi Y, Fujii T, Yoshikumi C, Nomoto K. (1986) Restoration of depressed immune responses by PSK in C3H/He mice bearing syngeneic X5563 tumor. *Japanese Journal of Cancer & Chemotherapy* 13:3453-2460
  88. Matsunaga K, Morita I, Oguchi Y, Fujii T, Yoshikumi C, Nomoto K. (1986) Competitive effect of PSK against the immunosuppressive effect induced in the sera of mice bearing syngeneic tumors. *Japanese Journal of Cancer & Chemotherapy*. 13:3461-3467
  89. Matsunaga K, Morita I, Oguchi Y, Fujii T, Yoshikumi C, Nomoto K. (1986) Restoration of immunological responsiveness by PSK in tumor-bearing animals. *Japanese Journal of Cancer & Chemotherapy*. 13:3468-3475
  90. Matsunaga K, Ohhara M, Oguchi Y, Iijima H, Kobayashi H. (1996) Antimetastatic effect of PSK, a protein-bound polysaccharide, against the BL16-BL6 mouse melanoma. *Invasion & Metastasis* 16:27-38
  91. Matsuzaki T, Nakashima Y, Ikegami M, Okubo H, Hino K, Emura T, Shinkawa G, Hirai K, Abe H, Tanikawa K. (1989) A four-year follow-up study of hepatocellular carcinoma with bone metastasis. *Japanese Journal of Cancer Clinics* 35:1673-1680
  92. Maxwell-Armstrong CA, Durrant LG, Scholefield JH. (1999) Immunotherapy for Colorectal Cancer. *American Journal of Surgery* 177:344-348
  93. Mayer P, Drews J. (1980) The effect of a protein-bound polysaccharide from *Coriolus versicolor* on immunological parameters and experimental infections in mice. *Infection* 8:13-21
  94. Mikuriya S, Hatano K, Konoeda K, Kamimura S, Sagara M, Oda T, Ooami H. (1987) Study of abscopal effect and cellular infiltration of tumor nests using less-fractionated, large-dose radiation. *Japanese Journal of Cancer Clinics* 33:1239-1252
  95. Mitomi T, Kyoji-Ogoshi. (1986) Clinical study of PSK as an adjuvant immunochemotherapeutic agent against gastric cancer. *Japanese Journal of Cancer & Chemotherapy*. 13:2532-2537
  96. Mitomi T, Tsuchiya S, Iijima N, Aso K, Suzuki K, Nishiyama K, Amano T, Takahashi T, Murayama N, Oka H. (1989) Randomized controlled study on adjuvant immunochemotherapy with PSK in curatively resected colorectal. The Cooperative Study Group of Surgical Adjuvant Immunochemotherapy for Cancer of Colon and Rectum. *Japanese Journal of Cancer & Chemotherapy*. 16:2241-2249
  97. Miyaji C, Ogawa Y, Imajo Y, Imanaka K, Kimura S. (1983) Combination therapy of radiation and immunomodulators in the treatment of MM46 tumor transplanted in C3H/He mice. *Oncology* 40:115-119
  98. Miyanaga O, Shirahama M, Ishibashi H. (1990) A case of remission in metastatic lung tumor from hepatocellular carcinoma after combined CDDP and PSK therapy. *Japanese Journal of Cancer Clinics*. 36:527-531
  99. Miyazaki T, Yadomae T, Sigiura M, Ito H, Fujii K. (1974) Chemical structure of antitumor polysaccharide, coriolan, produced by *Coriolus versicolor*. *Chemical & Pharmaceutical Bulletin*. 22:1739-1742
  100. Monma Y, Kawana T, Shimizu F. (1997) In vitro inactivation of herpes simplex virus by a biological response modifier, PSK. *Antiviral Research* 35:131-138

101. Morino M, Tsuzuki T, Ishikawa Y, Shirakami T, Yoshimura M, Kiyosuke Y, Matsunaga K, Yoshikumi C, Saijo N. (1997) Specific regulation of HSPs in human tumor cell lines by PSK. *In Vivo* 11:261-264
102. Nakagami Y, Minowa T, Tozuka K, Hiraoka Y, Chin H. (1983) Evaluation of multidisciplinary treatment of bladder cancer, especially in chemoimmunotherapy (ADM and OK-432) as a consolidation therapy. *Cancer Chemotherapy & Pharmacology*. 11 Suppl:S47-50
103. Nakajima T, Ichikawa S, Uchida S, Komada T. (1990) Effects of a protein-bound polysaccharide from a basidiomycetes against hepatocarcinogenesis induced by 3'-methyl-4-dimethylaminoazobenzene in rats. *Clinical Therapeutics* 12:385-392
104. Nakajima T, Inokuchi K, Hattori T, Inoue K, Taguchi T, Kondo T, Abe O, Kikuchi K, Tanabe T, Ogawa N. (1985) A multi-institutional study on postoperative adjuvant immunochemotherapy of gastric cancer. *Japanese Journal of Cancer & Chemotherapy*. 12:1850-1863
105. Nakajima T, Inokuchi K, Hattori T, Inoue K, Taguchi T, Kondou T, Abe O, Kikuchi K, Tanabe T, Ogawa N. (1989) Multi-institutional cooperative study of adjuvant immunochemotherapy in gastric cancer—five year survival rate. *Japanese Journal of Cancer & Chemotherapy*. 16:799-806
106. Nakazato H, Ichihashi H, Kondo T. (1986) Clinical results of a randomized controlled trial on the effect of adjuvant immunochemotherapy using esquinon and krestin in patients with curatively resected gastric cancer. Cooperative Study Group of Cancer Immunochemotherapy, Tokai Gastrointestinal Oncology Group. *Japanese Journal of Cancer & Chemotherapy*. 13:308-318
107. Nakazato H, Koike A, Ichihashi H, Saji S, Danno M, Ogawa N. (1989) An effect of adjuvant immunochemotherapy using krestin and 5-FU on gastric cancer patients with radical surgery (first report)—a randomized controlled trial by the cooperative study group. Study Group of Immunochemotherapy with PSK for Gastric Cancer. *Japanese Journal of Cancer & Chemotherapy*. 16:2563-2576
108. Nakazato H, Koike A, Saji S, Ogawa N, Sakamoto J. (1994) Efficacy of immunochemotherapy as adjuvant treatment after curative resection of gastric cancer. *Lancet* 343:1122-1126
109. Ng TB. (1998) A review of research on the protein-bound polysaccharide (polysaccharopeptide, PSP) from the mushroom *Coriolus versicolor* (basidiomycetes: polyporaceae) *Gen. Pharmac.* 30:1-4
110. Nguyen BT, Stadtsbaeder S. (1979) Comparative biological and antitoxoplasmic effects of particulate and water-soluble polysaccharides, in vitro. *Advances in Experimental Medicine & Biology* 121A:255-268
111. Niimoto M, Hattori T, Tamada R, Sugimachi K, Inokuchi K, Ogawa N. (1988) Postoperative adjuvant immunochemotherapy with mitomycin C, futraful, and PSK for gastric cancer. An analysis of data on 579 patients followed for five years. *Japanese Journal of Surgery* 18:681-686
112. Nishihara T, Hirayama K, Shineha R, Sanekata K, Shiga K, Takano R, Mori S. (1988) Improvement of treatment of carcinoma of the thoracic esophagus, especially in reference to the transition in postoperative combined therapy. *Journal of Japan Surgical Society*. 89:1479-1482

113. Nishihira T, Kasai M. (1984) Progress in postoperative care and postoperative combined therapy of esophageal carcinoma by total parenteral nutrition (TPN). *Journal of Japan Surgical Society*. 85:965-969
114. Nishiwaki Y, Furuse K, Fukuoka M, Ota M, Niitani H, Asakawa M, Nakai H, Sakai S, Ogawa N. (1990) A randomized controlled study of PSK combined immunochemotherapy for adenocarcinoma of the lung. The Advanced Lung Cancer Immunochemotherapy Study Group. *Japanese Journal of Cancer & Chemotherapy* 17:131-136
115. Nomoto K, Tsuru S, Awane Y, Kamei H, Ishibiki H, Sekiguchi M, Tamada R, Toge T, Nakajima T. (1986) Changes in nonspecific factors in the serum of gastric cancer patients after surgery and immunochemotherapy. *Japanese Journal of Cancer & Chemotherapy*. 13:458-463
116. Ogawa K, Hirai M, Katsube T, Kajiwarra T. (1993) Surgical stress and lymphocyte function: effects of PSK to prevent cellular immunosuppression. *Journal of the Nippon Medical School* 60:316-320
117. Ogawa Y, Kimura S, Imajo Y, Hamada F, Inomata T, Ichiyanagi A, Miyaji C, Imanaka K, Oshitani T, Takashima H, Ohbayashi K, Takada Y, Kono M. (1981) Clinical effect of concomitant use of PSK on 121 cases of primary lung cancer (Stage III, IV) treated with radiation combined with chemotherapy. *Journal of Japan Society for Cancer Therapy*. 16:713-723
118. Ogoshi K, Miyaji M, Nakamura K, Kondoh Y, Makuuchi H, Tajima T. (1998) Immunotherapy and combined assay of serum levels of carcinoembryonic antigen and acute-phase reactants. *Cancer Immunology, Immunotherapy* 46:14-20
119. Ogoshi K, Satou H, Isono K, Mitomi T, Endoh M, Sugita M. (1995) Possible predictive markers of immunotherapy in esophageal cancer: retrospective analysis of a randomized study. The Cooperative Study Group for Esophageal Cancer in Japan. *Cancer Investigation* 13:363-369
120. Ogoshi K. (1988) Evaluation of immunotherapy with PSK in esophageal cancer. *Japanese Journal of Cancer & Chemotherapy*. 15:3143-3151
121. Oguchi Y, Ando T, Matsunaga K, Fujii T, Yoshikumi C, Nomoto K. (1987) Effect of PSK on cytotoxicity against sarcoma-180 in tumor-bearing mice. *Anticancer Research* 7:681-684
122. Ohno R, Yamada K, Masaoka T, Ohshima T, Amaki I, Hirota Y, Horikoshi N, Horiuchi A, Imai K, Kimura I. (1984) A randomized trial of chemoimmunotherapy of acute nonlymphocytic leukemia in adults using a protein-bound polysaccharide preparation. *Cancer Immunology, Immunotherapy* 18:149-154
123. Ooi V, Fang L. (2000) Immunomodulation and anticancer activity of polysaccharide-protein complexes. *Current Medicinal Chemistry* 7:715-729
124. Ozaki S, Okazaki T, Nakao K. (1995) Biological response modifiers (BRM) as antigens: III. T cell lines specific for BRM kill tumor cells in a BRM-specific manner. *Cancer Immunology, Immunotherapy* 40:219-227
125. Pang Z, Chen Y, Zhou M, Wan J. (2000) Effect of polysaccharide krestin on glutathione peroxidase gene expression in mouse peritoneal macrophages. *British Journal of Biomedical Science* 57:130-136



126. Pang Z, Chen Y, Zhou M. (2000) Polysaccharide krestin enhances manganese superoxide dismutase activity and mRNA expression in mouse peritoneal macrophages. *American Journal of Chinese Medicine* 28:331-341
127. Papadopoulos-Eleopoulos E, Hedland-Thomas B, Causer DA, Dufty AP. (1989) An alternative explanation for the radiosensitization of AIDS patients. *International Journal of Radiation Oncology, Biology, Physics*. 17:695-697
128. Patchen ML, DiLuzio NR, Jacques P, MacVittie TJ. (1984) Soluble polyglycans enhance recovery from cobalt-60-induced hemopoietic injury. *Journal of Biological Response Modifiers* 3:627-633
129. Saito Y, Hori T, Takami M, Muraoka K, Hokama Y, Numata H. (1983) Study on postoperative local chemotherapy of malignant brain tumors using ACNU and PSK. *Japanese Journal of Cancer & Chemotherapy* 10:1963-1971
130. Saito Y. (1984) Long-term survival of patients with brain tumors treated with ACNU and PSK after surgery—with special reference to their immunological follow-up. *Japanese Journal of Cancer & Chemotherapy*. 11:2185-2192
131. Saji S, Kajima T, Takao H, Ban K, Ohashi H, Kunieda T, Sakata K. (1983) A study on postoperative long-term continuous immunochemotherapy with PSK and 5-FUDS for advanced gastric and colorectal cancers. *Japanese Journal of Cancer & Chemotherapy*. 10:756-762
132. Sakagami H, Ikeda M, Konno K. (1989) Stimulation of tumor necrosis factor-induced human myelogenous leukemic cell differentiation by high molecular weight PSK subfraction. *Biochemical and Biophysical Research Communications* 162:597-603
133. Sakagami H, Tsutomu A, Simpson A, Tanuma S. (1991) Induction of immunopotential activity by a protein-bound polysaccharide, PSK. *Anticancer Research* 11:993-1000
134. Sakurai K, Motokawa I, Ando T, Ohmura Y, Saito K, Matsunaga K, Fujii T, Yoshikumi C. (1993) Effect of a biological response modifier, PSK, on intestinal flora of tumor-bearing mice. *In Vivo* 7:175-179
135. Schneider SB, Nishimura RD, Zimmerman RP, Tran L, Shiplacoff J, Tormey M, Contreras R, Juillard GF. (1999) Filgrastim (r-metHuG-CSF) and its potential use in the reduction of radiation-induced oropharyngeal mucositis: an interim look at a randomized, double-blind, placebo-controlled trial. *Cytokines, Cellular & Molecular Therapy* 5:175-180
136. Sohma M, Kitagawa T, Okano S, Utsumi M, Mutoh E, Takeda S, Kanda M, Suzuki Y, Okamura K, Namiki M. (1987) Two cases of unresectable pancreatic cancer responding to combined chemotherapy with cisplatin, PSK, and UFT. *Japanese Journal of Cancer & Chemotherapy* 14:1926-1929
137. Sugimachi K, Miyauchi Y, Inutsuka S, Kakegawa T, Nishi M, Tomita M, Koga Y, Ishigami K, Inokuchi K. (1987) Effectiveness of multidisciplinary treatment combined with immunotherapy in esophageal cancer—analysis of 287 cases of carcinoma of the thoracic part of the esophagus in Kyushu and Yamaguchi districts. *Japanese Journal of Cancer Clinics* 33:777-783
138. Suzuki M, Watanabe T, Katakura R, Suzuki J, Mori T, Wada T. (1983) Combined radio-immuno-chemotherapy for medulloblastoma. [*Japanese Journal of Neurological Surgery* 11:1271-1276

139. Taguchi T. (1978) Clinical studies on PSK: combination therapy of PSK with radiation in cancer of the uterine cervix. *Recent Results in Cancer Research* 68:174-177
140. Takada M. (1983) Nonspecific cell-mediated immunity of gynecologic cancer patients and related effects of chemotherapy and immunotherapy. *Japanese Journal of Cancer & Chemotherapy*. 10:1480-1485
141. Takada T, Ashie T, Kikuri K, Hosono A, Ogayu T, Matsuki T, Hanawa N, Shoji T, Imura O. (1983) Case of hepatocellular carcinoma with a marked reduction in the tumor size induced by PSK administration alone. *Japanese Journal of Cancer & Chemotherapy*. 10:1530-1535
142. Takahata K, Yamanaka M, Oka H. (1985) Effect of PSK on prostaglandin metabolism. *Japanese Journal of Cancer & Chemotherapy* 12:1131-1136
143. Takashima S, Kinami Y, Miyazaki I. (1988) Clinical effect of postoperative adjuvant immunochemotherapy with FT-207 suppository and PSK in colorectal patients. Colorectal Cancer Chemotherapy Group in Hokuriku. *Japanese Journal of Cancer & Chemotherapy*. 15:2229-2236
144. Tamada R, Inokuchi K, Hattori T, Inoue K, Taguchi T, Kondo T, Abe O, Kikuchi K, Tanabe T, Nakajima T. (1987) A multi-institutional study on postoperative adjuvant immunochemotherapy of gastric cancer (II). *Japanese Journal of Cancer & Chemotherapy*. 14:716-722
145. Taniguchi M, Tsuru S, Kitani H, Zinnaka Y, Nomoto K. (1984) Depression of protective mechanisms against ectromelia virus infection in tumor-bearing mice and its prevention by PSK. *Japanese Journal of Cancer & Chemotherapy*. 11:2760-2765
146. Tochikura TS, Nakashima H, Hirose K, Yamamoto N. (1987) A biological response modifier, PSK, inhibits human immunodeficiency virus infection in vitro. *Biochemical & Biophysical Research Communications* 148:726-733
147. Tochikura TS, Nakashima H, Yamamoto N. (1989) Antiviral agents with activity against human retroviruses. *Journal of Acquired Immune Deficiency Syndromes* 2:441-447
148. Toge T, Kegoya Y, Yamaguchi Y, Baba N, Kuninobu H, Takayama T, Yanagawa E, Hattori T. (1989) Surgical stress and immunosuppression in cancer patients. *Japanese Journal of Cancer & Chemotherapy*. 16:1115-1121
149. Tomochika H, Gouchi A, Okanobu K, Sasaki A, Fuchimoto S, Orita K. (1989) The effect and distribution of a protein-bound polysaccharide preparation, PSK (krestin), intratumorally injected prior to surgery into gastric cancer patients. *Acta Medica Okayama* 43:289-297
150. Torisu M, Hayashi Y, Ishimitsu T, Fujimura T, Iwasaki K, Katano M, Yamamoto H, Kimura Y, Takesue M, Kondo M, Nomoto K. (1990) Significant prolongation of disease-free period gained by oral polysaccharide K (PSK) administration after curative surgical operation of colorectal cancer. *Cancer Immunology, Immunotherapy* 31:261-268
151. Tsuji K, Takagi M, Kobayashi T, Ishiguro A, Naganuma K, Koike K, Nakahata T, Akabane T. (1989) Effect of a protein-bound polysaccharide, PSK, on human hemopoietic progenitors. *Acta Haematologica Japonica* 52:594-600
152. Tsujimura S, Kato J, Ueda K, Ohtaguro K. (1983) Treatment of tumors of the urinary bladder with so-called immunopotentiators. *Acta Urologica Japonica*. 29:9-15

153. Tsukagoshi S, Hashimoto Y, Fujii G, Kobayashi H, Nomoto K, Orita K. (1984) Krestin (PSK). *Cancer Treatment Reviews* 11:131-155
154. Tsuru S, Nomoto K, Taniguchi M, Kitani H, Watanabe M, Zinnaka Y. (1984) Depression of macrophage functions and T-cell-mediated immunity to listeria infection in tumor-bearing mice and its prevention by PSK. *Cancer Immunology, Immunotherapy* 18:160-163
155. Tsuru S, Oguchi M, Mashiko M, Aiso S, Zinnaka Y, Nomoto K. (1982) Effect of PSK on tumor-specific immunity induced by MMC-treated syngeneic tumor cells. *Japanese Journal of Cancer & Chemotherapy* 9:1634-1639
156. Tsuru S, Oguchi Y, Morita I, Fujii T, Matsunaga K, Yoshikumi C, Hotta T, Nomoto K. (1983) Involution of the thymus in tumor-bearing mice and its restoration by PSK. *Journal of Clinical & Laboratory Immunology* 11:43-46
157. Tsuru S, Shinomiya N, Katsura Y, Gotoh M, Noritake M, Nomoto K. (1991) Effects of combined therapies with protein-bound polysaccharide (PSK, krestin) and fluorinated pyrimidine derivatives on experimental liver metastases and on the immunologic capacities of the hosts. *Oncology* 48:498-504
158. Tsuru S, Taniguchi M, Zinnaka Y, Nomoto K. (1984) Effect of PSK on the recovery of macrophage function and T-cell-mediated immunity in tumor-bearing mice. *Japanese Journal of Cancer & Chemotherapy* 11:2375-2380
159. Tsuru S. (1992) Depression of early protection against influenza virus infection by cyclophosphamide and its restoration by protein-bound polysaccharide. *Kitasato Archives of Experimental Medicine* 65:97-110
160. Uetsuka A, Satoh S, Ohno Y. (1979) Protective effect of PSK, a protein-bound polysaccharide preparation against candidiasis in tumor-bearing mice. *Advances in Experimental Medicine & Biology*. 121B:21-31
161. Umeda T, Kojima T, Kamei H, Kondo T. (1984) Leukocyte migration inhibition (LMI) of murine spleen cells after immunization with tumor cells, and an experimental study with LMI in combination therapy with a non-specific immunostimulant and chemotherapeutic agent. *Journal of Japan Surgical Society* 85:206-217
162. Urano M, Kuroda M, Reynolds R, Oberley TD, St. Clair DK. (1995) Expression of manganese superoxide dismutase reduces tumor control radiation dose: gene-radiotherapy. *Cancer Research* 55:2490-2493
163. Vallis KA. (1991) Glutathione deficiency and radiosensitivity in AIDS patients. *Lancet* 337:1616
164. Wakisaka S, Maruoka N, Kaji Y, Moriyama T, Oyama H, Oyama H, Shimauchi M, Nonaka A, Goya T, Kinoshita K, Kodama T. (1988) AUFRAp therapy: combined modality treatment of malignant gliomas with intraarterial infusion of ACNU. *Japanese Journal of Cancer & Chemotherapy*. 15:2405-2409
165. Wasser S, Weis A. (1999) Therapeutic effects of substances occurring in higher basidiomycetes mushrooms: a modern perspective. *Critical Review of Immunology* 19:65-96
166. Yagita A, Itoh H, Watanabe Y, Seki M, Katajima M, Tachikawa I. (1986) A long-term survivor of primary hepatoma—a case suggesting the superiority of a multi-drug (OK-432, SPG, PSK) over mono-drug therapy. *Japanese Journal of Cancer & Chemotherapy*. 13:1992-1995

167. Yanagawa T, Oguro M, Takagi T, Takenaga K. (1984) Direct antitumor activity of biological response modifiers (BRM) proven by an in vitro sensitivity test. Japanese Journal of Cancer & Chemotherapy 11:2155-2162
168. Yasutomi M. (1986) Surgery and adjuvant therapy of cancer of the large intestine. Japanese Journal of Cancer & Chemotherapy. 13:2307-2317
169. Yefenof E, Gafanovitch I, Oron E, Bar M, Klein E. (1996) Prophylactic intervention in radiation-leukemia-virus-induced murine lymphoma by the biological response modifier polysaccharide K. Cancer Immunology, Immunotherapy 41:389-396
170. Yokoe T, Iino Y, Takei H, Horiguchi J, Koibuchi Y, Maemura M, Ohwada S, Morishita Y. (1997) HLA antigen as predictive index for the outcome of breast cancer patients with adjuvant immunochemotherapy with PSK. Anticancer Research 17:2815-2818
171. Yunoki S, Tanaka N, Hizuta A, Orita K. (1994) Enhancement of antitumor cytotoxicity of hepatic lymphocytes by oral administration of PSK. International Journal of Immunopharmacology 16:123-130
172. Zhang H, Morisaki T, Matsunaga H, Sato N, Uchiyama A, Hashizume K, Nagumo F, Tadano J, Kitano M. (2000) Protein-bound polysaccharide PSK inhibits tumor invasiveness by down-regulation of TGF- $\beta_1$  and MMPs. Clinical & Experimental Metastasis 18:343-352
173. Zhou M, Chen Y, Ouyang Q, Liu S, Pang Z, Wan J. (1998) The effect of tert-butyl hydroperoxide on peritoneal macrophages and the protective effect of protein-bound polysaccharide administered intraperitoneally and orally. American Journal of Chinese Medicine 26:301-310
174. Zhou M, Chen Y, Ouyang Q, Liu S, Pang Z. (2000) Reduction of the oxidative injury to the rabbits with established atherosclerosis by protein bound polysaccharide from *Coriolus versicolor*. American Journal of Chinese Medicine 28:239-249

Figure 1:

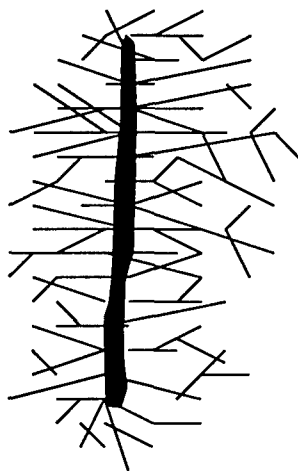


Figure 2:

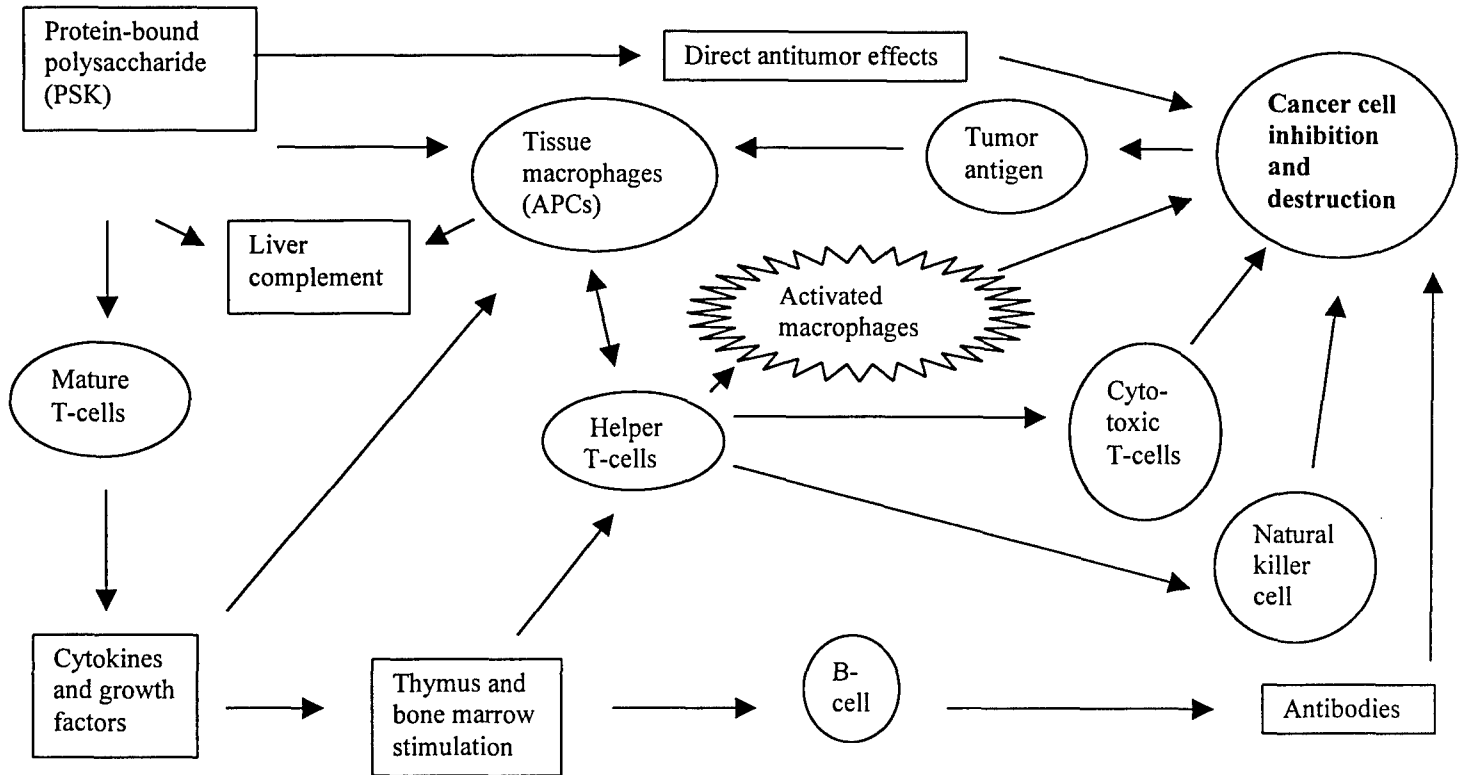
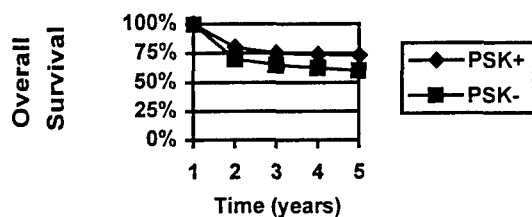
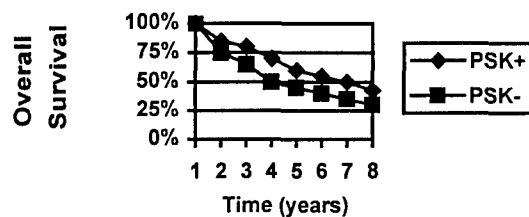


Figure 3 (a-g):

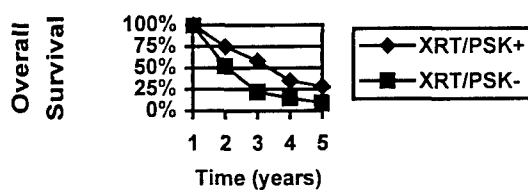
a: Survival in Gastric Cancer



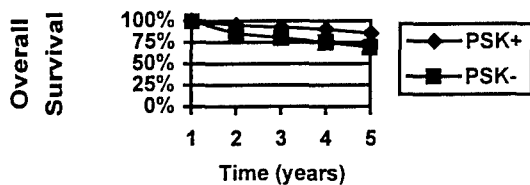
b: Survival in Colorectal Cancer



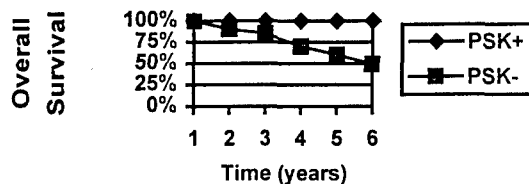
c: Survival in Non-small Cell Lung Cancer (NSCLC)



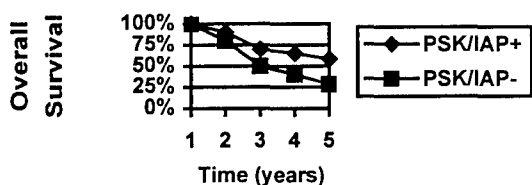
d: Survival in Breast Cancer



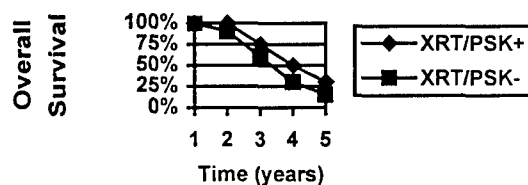
e: Survival in HLAB40+ Breast Cancer



f: Survival in Esophageal Cancer Stratified by IAP Levels



g: Survival in Nasopharynx Cancer





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REPLY TO  
ATTENTION OF:

MCMR-RMI-S (70-1y)

28 July 03

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